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HYPERSENSITIVITY AND IMMUNITY IN THE LIGHT OF THE "UNITARIAN" HYPOTHESIS*†

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I PRESUME that the suggestion that I discuss the "Unitarian" Hypothesis was motivated by the fact that in addition to the long-standing lack of unanimity of opinion on the question of identity of antibodies responsible for the increased resistance, on the one hand, and for increased sensitivity on the other (that is, classical antibodies versus "atopic reagins"), lately there has been raised another question, that of identity of the so-called "blocking" antibody (that is, "sensitizing" versus "neutralizing" antibody).

Since I addressed essentially the same group on the former subject nearly ten years ago, I will limit myself to a brief, more or less dogmatic restatement of my conclusions, and will use most of my time in an attempt to define the "Unitarian" Hypothesis itself and to inquire whether or not this hypothesis needs revision in view of the recent discovery of the "blocking" antibody.

When a substance capable of exhibiting immunogenic stimulus is introduced parenterally into a suitable host, such an exposure produces radical changes in the physiologic processes of the recipient. This alteration is specific and essentially consists of an acquisition of an alertness in the reactivity of the tissues, expressed by their increased capacity to immobilize, neutralize, and eventually eliminate the particular foreign antigen if it is reintroduced into such an altered organism. The specificity of this altered response is mediated by antibodies through their capacity of combining with the specific antigen.

In this basic reaction the union between the antigen and its specific antibody is accomplished by the attachment of antibody molecules to each of the specific receptive sites on the surface of the antigen, so that the particles of the antigen collect around themselves the molecules of antibody globulin. As a consequence of this basic specific union and depending on a variety of modifying factors (such as the relative proportions of antigen and antibody,[36] the relative location of the particular antigenic component in the architecture of the complex antigenic particle, the presence of complement, the compositions of the medium with respect to inorganic and organic constituents other than the specifically

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reacting components,[57] etc.), a variety of secondary reactions are set in motion. Some of these secondary reactions, when taking place in vitro, can be observed directly (precipitation, agglutination, swelling), while others can be detected only by indirect methods utilizing various indicators (such as a hemolytic system to detect whether or not the complement fixation has taken place, a suspension of leucocytes to show whether or not the antigen was opsonized or a living animal to determine whether or not a toxin or a virus has been neutralized, etc.).

In most instances the antigens consist of natural products of tissues of animal or plant origin. Their make-up is very complex and usually consists of several components, each possessing independent antigenic or haptenic properties. Thus, for instance, human erythrocytes possess at least ten known antigenic components, and even such relatively simple substances as horse serum represent a composite of at least four or probably six independent antigens.[54] Thus, when an animal receives parenterally a suspension of intact pneumococci, it produces several antibodies, each having a specificity corresponding to that of each respective antigenic component of this complex antigen. In such a case there is, of course, no doubt that these antibodies differ one from another inasmuch as they are directed in their specific activity toward different antigenic determinants of the pneumococcus.

The differences of opinion concerning the unicity or plurality of antibodies are related to an entirely different subject; namely, whether or not the different secondary reactions which one may observe as a result of the union of a *single* antigenic component with the specific antiserum are all due to a single antibody contained in this antiserum or whether different antibodies, each capable of exhibiting a particular function, are concerned in bringing about these different reactions. For instance, the type-specific antipneumococcal serum of a rabbit may contain several antibodies. It may have an antibody against the species-specific nucleoprotein of the cell, it may have another antibody reacting with a somatic carbohydrate, and it may still have another antibody reacting with the heterogenetic determinant entering into the composition of both the somatic protein and somatic carbohydrate of the cell; but such a serum will have still another antibody which specifically combines with the type-specific capsular polysaccharide.

When the latter antibody is allowed to react with intact cells or with specific polysaccharide isolated from them, (1) it will agglutinate intact pneumococci of homologous type; (2) it will cause swelling of these cells; (3) it will cause increased phagocytosis of homologous cells; (4) it will protect mice from infection by the cells of the same type; (5) it will precipitate the specific polysaccharide obtained from these cells; (6) it will cause fixation of complement in the presence of this specific polysaccharide, and (7) it will passively sensitize guinea pigs so that intravenous injection of a solution of this specific polysaccharide will cause an acute anaphylactic shock. The question is, Are these various reactions mediated by the same antibody or is each of them mediated by a different antibody?

In general, the type of evidence which is at the basis of the view that each of the reactions enumerated above is due to a different antibody is of two kinds.

It has been noted that a serum which exhibits several of the functions mentioned may lose the different functions upon dilution (or as result of heating) at different rates for each. For instance, in our example an immune serum, when progressively diluted, will cease to act as precipitin before it loses its power to act as agglutinin. Such a finding was, at one time, erroneously interpreted as indicating that antibody responsible for precipitation and the one responsible for agglutination are independent of each other and are present in the serum in different concentrations.[53]

Another type of evidence concerns qualitative differences exhibited by various antisera, particularly when obtained from animals of different species. For instance, while type-specific antipneumococcal serum produced in a rabbit will cause swelling of intact pneumococci of homologous type, will fix complement in the presence of the corresponding polysaccharide, and will passively sensitize a guinea pig to the polysaccharide, it was found that an antiserum produced in a horse will fail to exhibit every one of these functions, although, like the antiserum produced in rabbits, it will exhibit all the other functions such as agglutination, precipitation, opsonization, and protection.

Later studies have shown, however, that the *quantitative* differences mentioned earlier are explained by the degree of dispersion of the antigen employed in the respective reactions and are not due to differences in the antibody concerned. It may take several thousand times less antibody globulin to cover the specific determinant sites on the surface of a given number of intact bacteria, and thus cause their aggregation, than is needed to cover the very large surface represented by finely divided particles of the specific antigenic component which becomes exposed when the same number of bacteria are broken up to prepare a suspension of antigen for the precipitin test.[81, 109] And as for the qualitative differences between antisera produced in animals of different species, it was found that failure to exhibit certain of the reactions is due to the fortuitous differences in the composition of these sera with respect to various constituents, other than the antibody globulin, which interfere in one way or another with the secondary phase of reactions, although the basic union with antigen, which is the only reaction directly depending upon the specific properties of the antibody, always takes place.[57, 110]

Moreover, a similar effect (that is, the specific combination between antigen and antibody, which may not be followed by secondary changes or reactions) has been observed also with the antisera of any one given animal species under certain circumstances. For instance, in relatively rare cases it has been found that serum collected from the animal very early in the process of immunization may combine in vitro with the antigen in solution without precipitating it, and it was suggested that such *early* antibody reacts as if it were "monovalent."[52, 85] On the other hand, it is frequently found that when artificial immunization is continued for a relatively long time, the antisera collected late may differ in their functional properties from the antisera collected earlier. For instance, Goodner and Horsfall[45] have noted that while ordinarily the serum of horses immunized with type-specific pneumococcus passively protects normal mice against infection with the homologous organisms, if the immunization of horses is continued

for an inordinately long time, the protective power of the resulting antiserum may be weakened. Moreover, when larger amounts of such sera are employed in the protection tests, there may be no protection at all, and thus this inhibiting effect of the excess of antiserum may be likened to a "prozone" phenomenon observed in other reactions. Similar findings were described by others. For instance, Dingle found that serum of rabbits receiving many immunizing injections of meningococcus ceases to exhibit bactericidal effect, while earlier bleedings yielded actively bactericidal antisera.[95] Similar behavior of antisera was encountered also in chronic infections (Brucellosis)[47] or in long-continued exposure of Rh-negative mothers to the antigenic stimulation of an Rh-positive embryo.[86, 106]

It is easy to show that, in these examples, actually one is *not* dealing with *different* antibodies but with *the same antibody in a modified state.* When diphtherial antitoxin (which precipitates with toxin and neutralizes it) is photooxidized in vitro, it may lose its power to precipitate toxin, but its capacity to neutralize toxin is unaffected.[99] It is clear that in such an in vitro modification, the antibody retained its primary property of combining (neutralizing) with the toxin but lost its power to initiate a secondary phase of the reaction (precipitation). It is possible to show that the analogous modifications of antibodies, when they occur in vivo, also involve the failure of secondary reaction only. (1) When bacteria or erythrocytes, in the examples given, are exposed to nonagglutinating antisera, in spite of their failure to become agglutinated, the specific sites on the surface of these bacteria or erythrocytes evidently have been covered up or "blocked," since such cells will no longer be agglutinated by the respective ordinary agglutinating antibodies.[86, 106] (2) At least in the case of the failure of agglutination of Rh-positive erythrocytes, it was shown that this blocking effect is actually due to the union with antibody, for now, after exposure to such a nonagglutinating antiserum, the cells will be agglutinated by the serum of a rabbit immunized to human globulin, which proves that the surface of the cells has been coated by the antibody globulin* (and thus they act exactly as particles of inert collodion coated with globulin would act). (3) Most important, however, is the fact that if one merely changes the experimental conditions by suspending the cells of the respective antigens *in serum*, instead of in salt solution, or if to the suspension of cells in salt solution one adds a proper amount of albumin, such "blocking" or nonagglutinating antisera agglutinate respective antigens normally.[26, 47]

These latter observations are particularly significant in showing that the nonagglutinating or "blocking" antibody is *not* different but that it represents the same antibody in a somewhat modified state with respect to its capacity to initiate the secondary reactions. Just what is the cause of such a modification of antibodies and what are the conditions which may bring it about in vivo is not known, but some recent experiments suggest, for instance, that the route of introduction of antigen may be one of the determining factors. At least in horses, when identical antigen was injected, in accordance with identical schedule of injections, the animals receiving the antigen intravenously produced ordinary

*Coombs, R. R. A., Mourant, A. E., and Race, R. R.: Brit. J. Exper. Path. **26**: 225, 1945. See also earlier similar observations by Moreschi, C.: Centralbl. f. Bakteriol. **46**: 49, 1908.

precipitating antibodies, while those receiving the antigen subcutaneously or intracutaneously produced nonprecipitating antibody.[97] What other conditions, aside from the route of injection, may determine the production of modified antibodies is impossible to say. However, in vitro analogous modifications have been accomplished by means of a variety of procedures such as photo-oxidation,[98] chemical treatment,[40] extraction of lipids (reversible upon reintroduction of lipids),[49, 57] aging,*[56] agitation,[63] and exposure to heat.[69, 70, 94] While actual mechanisms responsible for the in vitro modification of antibodies effected by these procedures are not known, and may not be alike in all instances, at least in the case of transformation by heat the mechanism has been carefully studied.

The in vitro modification of antibodies under the influence of heat, according to Kleczkowski,[70] passes through two steps prior to the complete denaturation and loss of combining power. At first there occurs coacervation between the particles of antibody globulin and the nonspecific proteins of the serum. The complexes which result still exhibit original specific combining power, but their behavior with respect to the second stage of the reaction, such as flocculation, depends on the protein which participates in the formation of the complex. Coacervation of antibody globulin with normal euglobulin affects the flocculating power only slightly. But when antibody is heated in the presence of whole serum as a medium, "mixed complexes" are formed largely with serum albumin and some additions of other serum constituents, and these mixed complexes, while still combining specifically with the antigen, fail to flocculate it and, consequently, exhibit properties analogous to those of "blocking" antibody. The relative proportion of antibody globulin which entered into formation of the complexes to that remaining unchanged thus constitutes a competitive system and determines the over-all behavior of the modified antiserum.

Whether or not the similarly functioning modified antibodies, as they occur in vivo (as, for instance, when antigens are introduced subcutaneously,[97] or as result of long-continued exposure, as in isoimmunization with Rh-positive cells,[86] or in chronic brucella infection[47]), are due to the formation of similar complexes is not known, but such possibility seems worthy of consideration, particularly in view of recent findings that Rh-positive erythrocytes sensitized with corresponding blocking antibody (in addition to being agglutinable, as expected, by antiglobulin serum) are strongly agglutinated by the serum of rabbits immunized with purified human serum-albumin.† One need merely postulate that, under certain conditions, some highly avid constituents of the tissues or blood can coacervate with globulin, as has, in fact, been suggested by the findings of Majoor[80] from his cataphoretic data on serum fractions.‡

This possibility is further strengthened by the findings that under certain circumstances the blood may contain unusual protein constituents displaying great capacity of adsorption, which may simulate the behavior of specific antibodies when this adsorptive power happens to be preferential, as in the case of

*Probably the deterioration of "sensitizing" antibody on aging, noted by Walzer and Grove,[103] is due to the same process.

†Coombs, R. R. A., and Mourant, A. E.: J. Path. & Bact. 59: 105, 1947.

‡Also Morgan[84] found that cataphoretic data of hyperimmune rabbit serum indicated the presence of antibody in β globulin and albumin in addition to γ fraction of the serum.

anti-C protein of Abernathy and Avery,[2] and the substance described by Löfström as resembling anti-C protein which appears in the blood during the acute stage of many infections, and (especially suggestive) also in *normal individuals who receive parenteral injections of various antigens.** It is evident that somewhat analogous combinations must be taking place whenever any hapten is conjugated into an antigen in vivo (as in drug allergy).

However, formation of antibody complexes is not the only mechanism that may be thought of as a cause of formation of blocking antibodies. It is known that when highly complex antigens are introduced into an already sensitized organism (as is the case in pollen therapy), and, as a result, the more deeply seated somatic antigenic components are set free to stimulate production of antibodies with new specificities,[50, 51, 55, 107] the over-all behavior of the serum may be greatly modified.[64] It is conceivable that the different antibodies themselves may compete with one another for the neighboring sites on the surface of the antigen and cause steric hindrance sufficient in extent to interfere with secondary reactions.

It is conceivable that such a mechanism might be involved in connection with the blocking antibodies in erythroblastosis. There, the first antibody, which appears about the fourth month, reacts normally. Later, however, about the seventh month of pregnancy, the serum of the same patient may fail to agglutinate Rh-positive cells.[59]

Still another mechanism which might be responsible for the blocking effect of the sera secured after prolonged immunization may be that of production of "monovalent" antibodies which, by definition, combine with antigen but do not initiate the second phase of the reaction and thus, presumably, act as blocking antibodies.[108] The existence of monovalent antibodies has never been definitely proved, and although such experiments as the production of blocking antibodies from fully reacting antibodies, under the influence of photo-oxidation,[98] might be interpreted as representing the breaking of antibody molecules into monovalent fragments, the change can just as easily be due to formation of complexes similar to those described by Kleczkowski[70] as occurring under the influence of heat.

According to evidence presented in the foregoing discussion, it would seem apparent that the answer to the question as to whether or not the blocking antibodies are different from the usual ones, must be that they are identical inasmuch as they display the same specificity, and, in fact, any antibody can be transformed into a blocking antibody in vitro. However, those who consider the "neutralizing" (post-treatment) antibody to be different from the "sensitizing reagin" (pretreatment) state that in addition to its capacity to neutralize the allergen (capacity which is not displayed by the reagin) and thus to block the subsequent allergic skin reaction, the post-treatment antibody is more thermostable than is the nonblocking (sensitizing) pretreatment reagin.[76] It should be realized, however, that comparing the pretreatment serum of the hay fever sufferers with the post-treatment serum, we are dealing with two sera markedly dif-

*Löfström, G.: Acta Med. Scandinav. suppl. **141**: 1, 1942; also Brit. J. Exper. Path. **25**: 21, 1944.

fering in their antibody content. The pretreatment serum will have a relatively low total antibody content, and most of the antibody will be in unmodified ("sensitizing") form. The post-treatment serum will have considerably more total antibody and much of it will be in the modified form; that is, in the form which fails to initiate the secondary reactions ("blocking" or "nonsensitizing"). It is not surprising that upon exposure to the temperature at which all antibodies undergo gradual deterioration the pretreatment *(low titer)* serum will lose its capacity to sensitize sooner, without being intrinsically more sensitive to heat, simply because it will be transformed gradually into modified, nonsensitizing form, while the post-treatment serum, which already is in the modified form, will have to be completely denatured before it loses its only specific property of combining with antigen.

The need for a proper interpretation of the effects of heat upon antibodies is best illustrated in the case of alleged difference between antisera against H and O antigens. It has been stated by Jones[62] and confirmed by many workers that after twenty minutes at 90° C. the agglutinins against H antigen of hog cholera bacillus were not completely destroyed, while the anti-0 agglutinins were completely inactivated in twenty minutes at 75° C., and even at 65° C. the titer of the serum was reduced by one-half in twenty minutes. Careful investigations of Kleczkowski have shown that actually both antibodies are inactivated at a similar rate, but the residual portion of the unaltered (agglutinating) antibody is detected over a longer period of exposure to heat in the case of anti-H system because it requires less antibody for the agglutination due to the threadlike configuration of the antigenic molecules. Entirely analogous findings were obtained when antisera to plant viruses were compared. Antisera to tobacco mosaic virus (with rod-shaped particles, analogous to H antigens) lost their power to flocculate the specific antigen only when they were heated for ten minutes at 90° C., while antiserum to tomato bushy stunt virus (with nearly spherical particles, analogous to 0 antigens) lost its flocculating power after ten minutes of heating at 75° C.[6]

In the preceding discussion I have indicated the reasons which permit one to conclude that, while complex antigens may give rise to simultaneous production of several independent antibodies, *any one antigenic determinant* stimulates the production of a *single* antibody. I have indicated that the only specific activity of this antibody consists in uniting with the antigen.

The occurrence of this union in vivo, depending on the nature of antigen, speed, and route of entry, may be followed by a variety of secondary reactions which are quite important clinically. Similarly, when occurring in vitro, the consequences of the union may be ascertained by a variety of procedures, all of which depend on the combining activity of the same antibody (just as the presence and concentration of an electrolyte in the solution may be demonstrated through the determination of conductivity, boiling point, freezing point, and specific gravity, as well as by chemical analysis).

Due to fortuitous differences in the composition of the antibody-containing serum as a whole (as determined by the species of the animal, route and duration of immunization), possibly also due to a certain degree of heterogeneity between

the individual molecules of antibody globulin resulting from coacervation with other constituents of the serum, or due to imperfection in specific molecular configuration (thus involving their avidity), or due to incompleteness of their formation (which would involve their valence, etc.), some of the procedures may be ineffective in demonstrating the union; however, as long as the union, as demonstrated by any one of these procedures, involves the same specific determinants, it is clear that we are dealing with one antibody. This, in my understanding, is the meaning of the "Unitarian" Hypothesis.

Because the early investigators were impressed by the fact that, by the production of specific antibodies, the organism acquired the capacity of ridding itself of foreign material which frequently was injurious to the host, this process has been viewed as a protective mechanism and was referred to as "immunity," and antibodies as "protective."

However, this teleologic concept of the reaction on the part of an allergic* (modified by the previous exposure) animal organism to the parenteral introduction of foreign specific antigen did not adequately fit all the phenomena observed. For, while it is generally true that repeated parenteral exposure to toxins or to pathogenic bacteria may eventually render the organism relatively resistant (immune) to these substances, experience has shown that under certain conditions the repeated entry of even totally innocuous antigens into a previously exposed organism may produce severe symptoms or even death.

To explain this paradox, some investigators have suggested the possibility of the existence of two kinds of antibodies (one conferring the heightened resistance and the other, the heightened susceptibility), but, in view of the lack of evidence to support it, this hypothesis has been given up. The bulk of later evidence indicated that the *same* antibodies are responsible for the phenomena of both specific hypersensitiveness and specific resistance. However, there was still no agreement as to the nature of the mechanism determining which of these apparently widely different responses is to take place in a given instance. A widely accepted hypothesis was proposed that "the difference between the anaphylactic and immune state is quantitative rather than qualitative, depending on *distribution* of antibody."[96] According to this concept, "the anaphylactic state is associated with the presence of fixed antibody and the absence of circulating antibody," and "the immune state is associated with the presence of circulating antibody in a concentration sufficient to protect the fixed antibody that is also present."[96] That is to say, the presence of an excess of circulating antibody confers resistance (immunity) upon the sensitized animal because the antigen, by combining with circulating antibody, is prevented from reaching the sensitized tissues.

However, the observed facts do not support such a hypothesis.† That high concentration of circulating antibody does *not* protect the animal against anaphylactic shock is best illustrated by the well-known observation that immune

*This term is used here, as originally intended by von Pirquet, to denote the whole range of specifically altered reactivity: hyperergy, hypersensitiveness, anaphylaxis . . . or diminished reactivity: hypoergy, tolerance or immunity. (von Pirquet: Allergie, Berlin, 1910, Julius Springer, p. 86.)

†Morris, Marion C.: The Relation Between Antianaphylaxis and Antibody Balance, J. Exper. Med. 64: 641, 657, 1936.

animals with high antipolysaccharide antibody content in their blood (so high that a fraction of a cubic milliliter of their serum will passively protect mice against many thousand lethal doses of pneumococcus) are killed by anaphylactic shock if a mere trace of a specific polysaccharide in solution is injected intravenously.

Furthermore, if one were to assume that excess of antibody binds all of the injected antigen before it can reach the sessile antibody at the surface of the cells, one would have to postulate that the circulating antibody possesses greater avidity for the antigen. Yet when antigen is injected in small doses for the purpose of "desensitization" and thus, presumably, saturates the sessile antibody (and exhausts the capacity of cells to unite with subsequently introduced antigen), it is necessary to postulate the opposite; namely, that the sessile antibody has a greater avidity and can pick up the injected antigen in competition with the circulating antibody. Moreover, if desensitization saturates the combining power of the sessile antibody and thus prevents further uptake of antigen by the tissues at a later exposure to a *moderate* challenging dose, how can one explain the fact that such "desensitized" tissue reacts violently to a relatively larger challenging dose of antigen?

Neither can one explain, on the basis of the cellular hypothesis, the fact that in cases of multiple sensitivity desensitization with one of the antigens at fault (which, presumably, should exhaust only the specific sessile antibody) renders the sensitive subject nonreactive to an *equivalent* challenging dose of any other antigen to which it may be sensitive.[9, 24, 42, 82]

These and several other considerations[23] of equal weight demonstrate the inadequacy of the "cellular" hypothesis in explaining how the same antibody can bring about the increased resistance and the increased susceptibility.

Therefore, the search for a more satisfactory explanation of this paradox was directed back to a possibility that hypersensitivity may be due to the elaboration of a *special* antibody essentially *different* from that which is responsible for the specific resistance.

It has been noted that while in some cases of clinical allergy one can demonstrate by the usual procedures the presence of classical antibodies, quite frequently this is not the case. Furthermore, since the sensitivity in many such cases could not be traced to a known sensitizing exposure and since frequently the state of clinical hypersensitiveness of one kind or another exhibited a tendency to run in families, it was assumed that such sensitivity was acquired hereditarily and not as the result of previous sensitizing exposure, particularly since, in some instances, this sensitivity was directed toward exciting agents which were not antigenic in nature. It was suggested that the substance which determined the specificity of these responses was not a true antibody but a determinant of a different nature capable of being passively transferred (to normal human skin) but, unlike antibodies, not capable of eliciting the in vitro reactions with the allergen at fault. This determinant was given the noncommittal name of "reagin," and the hypersensitivity of this sort was called "atopy."[30] Subsequent studies have brought out several additional peculiarities of "reagins" which presumably differentiated them even more completely from the usual antibodies.[28]

This concept of spontaneously appearing (without previous exposure), hereditary hypersensitiveness (atopy) has received wide acceptance which has lasted to this day in spite of the fact that *each* of the peculiarities ascribed to "reagins" have been shown to be invalid, so that today there exists no need of differentiating between reagins and antibodies. Because of the confusion which results from unnecessary multiplication of terminology, it seems that the suggestion of Cooke[32] to abandon the hereditary feature of the concept of atopy should be extended to all other features of this concept (including the feature of *spontaneity* of atopic sensitivity) which attempt to differentiate atopy from classical specific hypersensitiveness of men and animals, resulting from a previous exposure and mediated by classical antibodies.[23]

Before closing this brief review of various antibodies and antibody-like specific determinants which have been implicated in the phenomena of hypersensitiveness and offering a concept which might explain the paradox we are considering on the basis of a "Unitarian" Hypothesis, it is necessary to consider the alleged role of still another (blocking) type of antibody in these phenomena.

Although the general attention to the possible role of blocking antibodies in allergic phenomena was aroused only recently, mainly by the studies of Cooke[33] and by others, the existence of these antibodies was recorded many years earlier by van Leeuwen and Kremer.[100] They found that the serum of allergic individuals, in addition to the "sensitizing" antibodies, often contains also a substance ("antiallergen") which inhibits the skin response to allergen. In fact, they calculated that 1 ml. of the serum of such an allergic patient can "bind," or neutralize, 0.1 ml. of a specific extract, so that approximately 5 liters of patient's total blood should be able to neutralize as much as 500 ml. of such an extract, and yet such patient may respond by a severe general reaction to subcutaneous introduction of very moderate doses of allergen. Quite correctly the authors predicted that injection of even a fraction of a milliliter of an allergen *intravenously* would probably kill such a patient in spite of the presence of large amount of "antiallergen" in the blood. They concluded, therefore, that the "antiallergens," or, as they are called now, "blocking antibodies," play no significant part in the establishment of an antiallergic (refractory) state.

To be sure, van Leeuwen was discussing the "antiallergens" found in the blood of allergic individuals *not* receiving any specific therapy, but there is no reason to suspect that these antibodies differ in any way, except in their titer, from those appearing in the course of a specific therapy.

That specific therapy may bring about notable clinical improvement is not surprising, since fundamentally it does not differ from the so-called "desensitization" procedure employed to minimize or prevent allergic reactions to relatively large doses of antigen both in men and in animals.* In fact, the value of specific therapy has not been seriously questioned through the thirty years since its introduction. However, it is only in the last few years that it has been claimed that this therapeutic procedure "brings about relief of symptoms *by the development of an antibody* which neutralizes the antigen at fault,"

*Besredka, A., and Bronfenbrenner, J.: Vaccination par petites doses, Ann. de l' Inst. Pasteur, 25: 393, 1911.

and thus prevents it from reacting "with its specific skin-sensitizing antibody in the blood or tissues."[3, 4] Furthermore, noting that massive "cumulative doses of antigen given to sensitive individuals is not enough to neutralize even one-tenth of the circulating reagin"[29] and that the concentration of reagin may actually increase as result of such procedures,[74] some investigators concluded that "only one other conceivable explanation" of the effectiveness of the repeated injection of allergens "is the formation of *neutralizing* immune antibodies."[29] This view is widely held today in spite of the earlier findings to the contrary of van Leeuwen and Kremer,[100] and in spite of the more recent evidence* that in many treated patients the titer of the blocking antibody may be found to be high without the concomitant clinical improvement and, *vice versa*, that clinical improvement may not be accompanied by a corresponding rise in blocking antibody titer.[11, 31, 43, 77, 93]

In view of the latter findings it would seem clear that the establishment and maintenance of a refractory state and the clinical improvement resulting from injections of related antigens are achieved by some other mechanism[24] and that the appearance of "neutralizing" (blocking) effects of antibody is not instrumental in establishing the refractory state but is *only incidental* to it.

It suffices to recall that the state of relative refractoriness can be established both in hypersensitive animals and in man within *minutes* and certainly can be made more effective within a *few hours* as a result of gradually increased doses of antigen given at short intervals, a period entirely too short for the production of any detectable amount of *new* antibodies. It is evident that whatever may be the mechanism capable of preventing the explosive consequences of the union between antigen and antibody, this mechanism must be mobilized immediately following the first "desensitizing" injection and must be fortified just as promptly by each succeeding one (as in "desensitization" in connection with serum therapy). Furthermore, if the refractory state following the repeated injections of antigen were due to the neutralizing effect of the blocking antibody, it should continue for the lifetime of these antibodies, which, in the case of any actively produced antibodies (and in the absence of further antigenic stimulus), means a period of four to six weeks at the least, and it should be strictly specific. The fact is, however, that the refractory state in actively sensitized animals lasts only a short time, usually not more than twenty-four hours (unless it is prolonged by further injections of antigen). Moreover, the refractory (desensitized) state is *not* specific. For instance, in multiply sensitized animals or individuals, the desensitization, carried out with either of the allergens at fault, is reciprocal when concentrations of *equal challenging value* (skin reactivity) are employed, and if the injection of allergens of unequal challenging value is employed, the stronger one desensitizes against all the others; only the weaker one produces what appears to be a specific unilateral desensitization.[9, 20, 24, 42, 82]

There is no reason to doubt, of course, that the failure of eliciting an allergic response by the sensitized skin to an injection of allergen combined with post-treatment serum is due to a blocking or neutralizing effect of the latter. However, there is no valid evidence that any *new* and *different* antibody is involved

*Alexander, H. L.: Personal communication.

in this phenomenon. On the basis of what was stated concerning the probable nature of blocking antibodies in general, one may be allowed to speculate that the post-treatment serum merely represents a mixture of some of the original antibody (reagin) with an excess of the same antibody that has been "modified" through the formation of "complexes" during prolonged immunization.* Such a serum may now exhibit combining power without being able to elicit secondary reactions and thus acts as a blocking antibody in preventing the union between the allergen (whose specific sites are *now covered* by modified antibody globulin) and the unmodified antibody of the sensitive skin.

If the neutralizing effect of the post-treatment serum is explained in this way, it also may be possible to understand why the sites receiving the mixture of pollen and post-treatment serum become fully reactive again within very few hours[48, 76] without postulating, as has been suggested, that in addition to other peculiarities the blocking antibody possesses also the unusual property of high diffusibility so that it promptly escapes from the site into adjacent tissues and, therefore, its local effect disappears in such a short time.[31, 76]

There is some evidence suggesting that the process of formation of "complexes" may be a reversible one and, if that were so, then at the moment when the antibody is set free from the complex, it immediately acts as a normal (sensitizing) antibody and the rate of diffusion need not be involved at all in explaining the rapid return of the sensitivity of the injected skin site.

That the postulate of reversibility of the coacervation is justified is suggested by the findings of Follensby and Hooker,[41] that even in vitro the blocking effect of nonprecipitating antibody shows a tendency to disappear in time, and the experiments of Morgan[84] suggest similar "reversal" as the result of dilution. It is to be expected that this reversal goes on at a higher rate in vivo than it does spontaneously on standing in the test tube, due to the action of enzymes, as suggested by the finding that nonprecipitating antibody complex promptly recovers its capacity to precipitate normally as result of exposure to pepsin[71] and probably to the other proteolytic enzymes.

The evidence presented in the preceding discussion suggested that in the establishment of the state of hypersensitiveness we are not dealing with any special or different antibody (reagin) and that desensitization is not due to exhaustion of sessile antibody nor to the production of new, blocking antibody.

But if that is so, what, then, is the mechanism which determines whether one and the same antibody will, in one instance, cause establishment of a relative heightened resistance and, in another instance, an increased sensitivity? And how does it happen that repeated introduction of small amounts of antigen increases the tolerance of a hypersensitive organism to the relatively large challenging doses of the antigen?

On the basis of a study of the phenomena of parenteral digestion,[18] a conclusion was reached that fundamentally there is no essential difference between the phenomena of increased resistance and increased susceptibility. In fact, they both represent different manifestations of a single biologic process set in

*That procedures which modify antibodies so that they become nonagglutinating, or nonprecipitating, may also deprive them of their power to transfer sensitivity and to acquire the power to block allergic reactions was shown at least in the case of one such procedure, photooxidation.[99]

motion by the union of a single antibody with its specific antigen. The first, phenomena of resistance, record the effects of the total process in so far as it involves the integrity (toxicity, capacity to invade) of the antigen; the second, symptoms of hypersensitivity, serve as an index of the changes involving the tissues of the host, both changes occurring in a single event *whenever* the antigen is reintroduced into the previously exposed organism, which now possesses a specific antibody and, therefore, reacts differently (= allergic[101] = immune = sensitized) from a normal, previously unexposed organism.

Basically this modified (allergic) response of the previously exposed (and now immune or sensitized) tissue consists of an inflammatory reaction which differs from the ordinary inflammation only by its rapid onset and a relatively stormy course. This inflammatory process is accompanied by both local and systemic symptoms closely resembling those elicited by the administration of histamine.

The outward expression of this injury to the host may *vary in intensity,* depending on the amount and nature (especially solubility) of the antigen as well as on the site and rate of its introduction into the sensitized organism.[22]

In final analysis, the availability of antigen for a *rapid* combination with specific antibody determines the intensity of this injury, and if conditions permit the combination to take place explosively and to involve vital structures (as the smooth musculature of the bronchioles when soluble antigen is introduced intravenously into a sensitized guinea pig), death may result before the concommitant immobilization of the antigen may become apparent.[23] Thus, for instance, animals possessing a high concentration of antitoxin in the blood may die of anaphylaxis as a result of introduction of very small amounts of purified toxin, although the same antitoxin which is responsible for the shock may be fully sufficient to prevent the intoxication.[68]

Under natural conditions of exposure, antigens enter tissues of allergic animals and of human beings slowly and in extremely small amounts; consequently, their union with antibodies is not explosive and the injury to the host is mild. The actual symptoms which are thus elicited vary in form, depending on the portal of entry and the amount of antigen and on the relative vulnerability of exposed structures, but the basic mechanism underlying all these reactions is identical with that which is responsible for the explosive experimental anaphylaxis[23] resulting from willful introduction of relatively large amounts of soluble antigens intravenously.

As long as the intensity of this process is such that the incidental injury to the host remains within the limits compatible with the essential physiologic processes, the total reaction results in checking the penetration of the antigen and is interpreted as protective in spite of concomitant transient injury to the host. But if secondary factors so modify the course of the reaction that injury to the host becomes incompatible with maintenance of essential life processes, we interpret the total response as that of increased susceptibility (anaphylaxis).

As a crude analogy, one might consider the process of burning wood in a hearth. As long as the fire is kept within the confines of the firebox and gives us comfortable warmth, we consider the process beneficial, disregarding the fact

that the loss of wood is inseparably associated with it. Occasionally, however, when the fire gets out of control and burns up the entire building, although heat was generated here just as before, we consider the event as destructive.[21]

The intimate mechanism by which the union between antigen and its specific antibody initiates these secondary reactions which, on the one hand, result in a more effective disposal of antigen and, on the other, a variable degree of injury to the host is not completely known even today. However, the accumulating evidence seems to validate basic postulates of a hypothesis[18] which I presented over thirty years ago at the Second Pan-American Scientific Congress. According to findings which served as a basis for the hypothesis[18] the circulating blood contains proteolytic enzymes,* the activity of which is normally kept in check by the inhibitors ("antitrypsin").[1, 13, 14, 46] The interaction of antibody with its specific antigen is accompanied by lowering of "antitryptic titer" of the blood and thus permits a temporary activation of serum-protease to take place and thus initiates autodigestion of the serum protein.[13, 15, 25] Either the active enzyme itself or some of the products of its activity (peptones) injure the tissues. If this reaction is carried out in vitro and the products are reinjected into the animals of the same species intravenously, the symptoms which follow immediately are indistinguishable from those of anaphylaxis.[12, 15, 16]

At the time these findings were recorded no information as to the exact nature of this injury was available. Subsequent studies, especially in the last five years, yielded information which gives insight into the probable nature of this tissue injury.[27, 78, 88] Thus it was found that injection of trypsin, or of peptone, liberates from the tissues preformed histamine[34, 37, 88, 89] which, as shown previously by Dale and Laidlaw,[35] reproduces most of the symptoms of anaphylaxis.

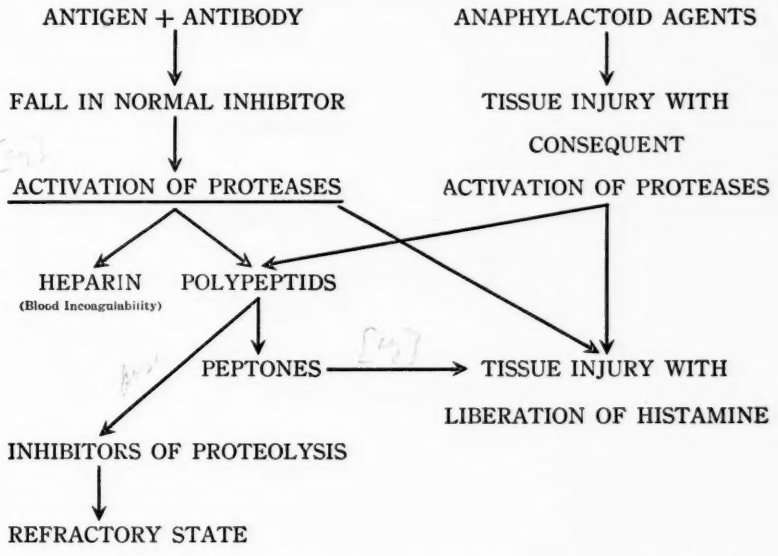
Furthermore, it was found that the union of antigen and antibody in vitro, in the presence of blood cells,[38,65, 67] skin,[66] and smooth muscle,[87] leads to liberation of preformed histamine. In view of these findings, I have attempted a few years ago[24] to bring together the earlier findings and those just cited into a single harmonious concept, which is represented in the accompanying schematic outline.

According to this concept the union between antigen and antibody lowers the inhibiting effect of antienzyme, thus setting free the enzyme. As shown by Rocha e Silva† the activity of this free enzyme is made possible by the kinase derived from the disintegrating platelets. Either the activated enzyme itself, or some of the products of its activity (peptones), injure tissue cells and set free from these cells the preformed histamine, which, in turn, causes the symptoms. Similar changes may occur also when proteolytic enzymes are set free by nonspecific means,[83, 91] causing the so-called anaphylactoid reactions. The only difference between the mechanism of true anaphylaxis and that of anaphylactoid reaction resides in the nature of the "trigger" which causes the activation of the enzyme; in specific anaphylaxis, the antigen-antibody union provides the "trigger," while in the anaphylactoid reactions, the "trigger" is a nonspecific

*In view of the uncertainty concerning the identity of the proteases[8] of the blood the terms trypsin and antitrypsin used earlier have been replaced in this discussion by the less commital terms protease and antiprotease or antienzyme, respectively.

†Rocha e Silva, M., Porto, A., and Androde, S. O.: The Role Played by Leucocytes and Platelets in the Genesis of the Shock, Arch. Surg. 53: 199, 1946.

injury, but the result in either instance is the liberation of the enzyme which, in turn, effects liberation of preformed histamine.[90] At the same time, the peptonelike toxic products of proteolysis, through their effect upon the liver,[60, 61, 104, 105] may cause the appearance of sufficient amount of heparin to slow down the rate of coagulation of the blood.*



The concept, as outlined above, also suggests the reason for the refractory state observed in sensitized animals surviving the injection of sublethal dose of antigen. Because of the reversible nature of the digestive process,[7] when the serum protease is activated following injection of a sublethal dose of antigen, the products of digestive process (polypeptides[72] and possibly other substances)[5, 39, 73, 92] act as inhibitors and delay or even arrest the further activity of the enzyme[17, 23] for a limited period (until these inhibitors are eliminated). Irrespective of the procedure by which the rise in the concentration of an inhibitor was produced, whether specifically (as a result of antigen-antibody reaction)[19, 25] or nonspecifically (as result of injection of anaphylactoid agents),[18, 91] the animals are found to be refractory to reinjection of *moderate* doses of antigen during the period while the inhibitor concentration is high. However, if the amount of antigen injected is so large that its union with excess of antibody permits liberation of enzyme in excess of that which can be bound by the inhibitor present, the proteolysis does occur and is followed by symptoms due to liberation of histamine.

In summing up this discussion, I would like to restate that the "Unitarian" Hypothesis is a concept which recognizes that complex antigens, possessing several independent specific determinants, give rise to several different antibodies with specific affinity for each determinant. However, in response to any one antigenic determinant, there is produced only one antibody which can be recognized by a variety of procedures, some direct and others indirect. Any given

*This is the only important accompaniment of the experimental anaphylaxis which is not expected to be duplicated in the shock produced by the injection of histamine.

single antibody may be unhomogeneous with respect to individual molecules of antibody-globulin. Some may possess both the combining power and the capacity to initiate secondary phenomena; others may have the same specific combining power but are imperfect in the sense that, for one reason or another (possessing a single valence only, or exhibiting diminished avidity, or low degree of specificity due to imperfect replication of antigenic determinants, or having entered into complex with other serum constituents, and possibly for other reasons), they are unable to initiate the secondary phenomena.

According to "Unitarian" Hypothesis, the phenomena of specific heightened resistance and heightened sensitivity are different expressions of a single biologic process: allergy; the first, records the consequences of the union of antigen with antibody as they affect the antigen; the second is an index of consequences of the same process as they affect the tissues of the host. Which of the sets of consequences attain dominance in a total observable reaction is determined by secondary factors insofar as they control the rate and total extent of these changes.

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HISTAMINE ANTAGONISTS

XI. Aerosolized Antihistamine Drugs in Prevention of Histamine Bronchospasm in Guinea Pigs*†‡

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OUR interest in experimentation on aerosols of antihistamine drugs in guinea pigs arises from the following considerations: (1) One of the probable explanations for the ineffectiveness of antihistamine drugs orally in asthma is that an insufficient concentration of the substance reaches the bronchial tissues. (2) The local application of the drug by aerosol may be more effective because this concentration may be increased. (3) Some degree of encouragement has been obtained in man in preliminary trials with aerosols of antihistamine drugs in asthma.[1]§ (4) The inhibiting effect of the drug aerosol on histamine bronchospasm in the guinea pig might prove to be a simple and reliable method of assaying antihistamine drugs, with a special advantage in saving of animals and a comparison of the same animals if a nonfatal endpoint were used.

PROCEDURE

In the experiments to be described we aimed at the following points: (1) To determine the effective range of concentration of the aerosol; (2) to compare the degree of effectiveness of different drugs; (3) to time the duration of effectiveness of the aerosols; (4) to observe their toxic or irritative actions; and (5) to compare these actions of the drugs qualitatively and quantitatively with other methods of administration, particularly intraperitoneal.

We conducted a number of preliminary experiments, varying the histamine concentration, the amount of air pressure employed in aerosolizing the drug and the histamine, the duration of exposure to the aerosols; studying the difference between continuous and discontinuous aerosolization of histamine; experimenting with various types of nebulizers; and determining the end points of the histamine effects on the animals. The details and findings of these preliminary trials are too lengthy to record here. We merely wish to indicate that these experiments resulted in the basis of our choice for the technic which we are to describe.

Separate aerosol chambers were used for the drug aerosol and the histamine aerosol. An air pressure of 5 pounds was used with both systems. Vaponephrin and De Vilbiss No. 40 nebulizers were employed to aerosolize the solutions. The drug aerosol chamber was prepared by aerosolizing the drug solution for five minutes. At the end of that time two or three guinea pigs were placed

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§Hamburger and his associates[2] tried aerosols of antergan and neoantergan in asthma and were not favorably impressed with the results.

in the chamber and the aerosolization continued. The animals were exposed for five minutes to this aerosol which varied in concentration from 4 per cent to 0.125 per cent. Fifteen minutes after the animals were removed from the drug aerosol they were placed in the second chamber which had had histamine* (0.5 mg. base per c.c.) aerosolizing into it for five minutes. The histamine aerosol was continued and the animals were removed as soon as labored breathing was observed.†

If none was present, the animal was left in the chamber a maximum time of ten minutes. In each single experiment ten animals were used, their normal reaction to histamine aerosol having been determined previously. Every few days this control response to histamine was checked and if the animal was not satisfactory it was replaced. The effect of a single concentration of a drug was repeated usually several times, because during the course of several months it was necessary to form new groups of animals. Only animals which responded normally with marked dyspnea to histamine in less than three minutes were chosen for these experiments.

The duration of the effect of the aerosol was studied by subjecting a number of animals to an effective concentration of the drug aerosol and then testing two to four animals at intervals of fifteen minutes, with the histamine aerosol. In a limited number of experiments we also attempted to study the comparative effectiveness and duration of action of intraperitoneal injection of several drugs on the histamine aerosol bronchospasm.

Our choice of the end point of dyspnea from histamine exposure needs some comment. We realize that death is an absolute unquestioned criterion of effectiveness. However, this had several objections: (1) The same animal cannot be used as a basis of comparison with other phases of experimentation; (2) it has the objection that other nonphysiologic methods have, that the conditions are too far removed from human clinical manifestations; (3) There is a sacrifice of many animals. Our method has as its major objection the fact that considerable experience and agreement are needed to determine the end point.

RESULTS

Effectiveness of Aerosols.‡—The results of these experiments are summarized in the accompanying graphs. They are expressed in terms of the number

*Histamine diphospate (Burroughs Wellcome & Co.) was used.

†Halpern[3] and most experimenters[4] use either death or convulsions as an end point.

‡The following antihistaminic drugs were used as aerosols:

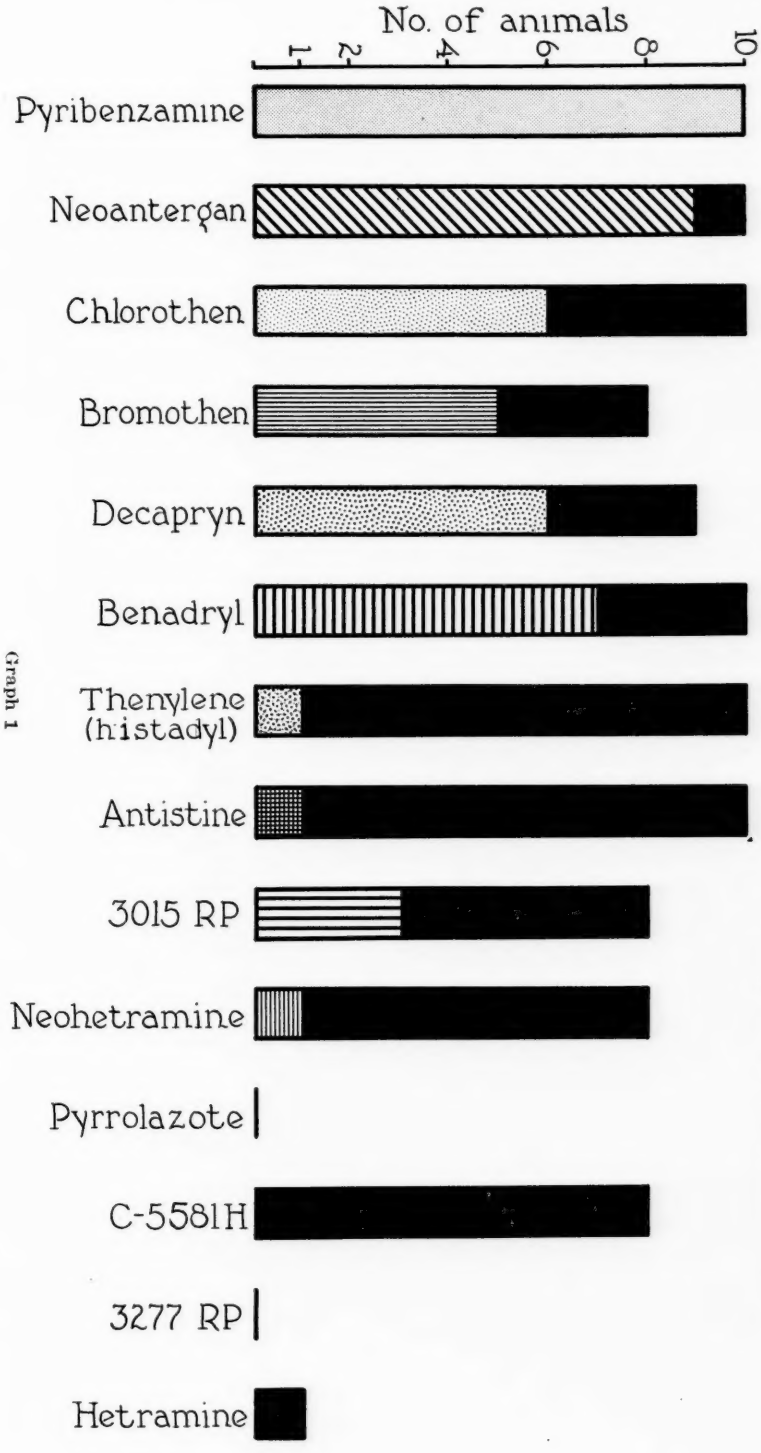
1. Pyribenzamine = N'pyridyl-N'benzyl-N-dimethylethylenediamine hydrochloride.
2. Neoantergan = N-p-methoxybenzyl-N-dimethylaminoethyl a aminopyridine maleate.
3. Chlorothen = N,N-dimethyl-N'-(2-pyridyl)N'-(5-chloro-2-thenyl) ethylenediamine hydrochloride.
4. Bromothen = N,N-dimethyl-N'-(2-pyridyl)N'-(5-bromo-2-thenyl) ethylenediamine hydrochloride.
5. Decapryn = Dimethylaminoethoxy-methylbenzyl-pyridine succinate.
6. Benadryl = B-dimethylaminoethyl benzohydryl ether hydrochloride.
7. Thenylene (Histadyl) N-(a-pyridyl)-N-(a-thienyl)-N',N-dimethylethylenediamine hydrochloride.
8. Antistine = 2-(N-phenyl-N-benzyl-aminoethyl)-imidazoline hydrochloride.
9. 3015 RP = N-dimethylaminoethyl thiodiphenylamine hydrochloride.
10. Neohetramine = 2-(N-dimethylaminoethyl-N-p-methoxybenzyl)-aminopyrimidine hydrochloride.
11. Pyrrolazote = N-B(-1-pyrrolidylethyl)phenathiazine hydrochloride.
12. C-5581 H = B-dimethylaminoethyl 2-benzylphenyl ether hydrochloride.
13. 3277 RP = N-dimethylaminopropyl thiodiphenylamine hydrochloride.
14. Hetramine = N,N-dimethyl-N'benzyl-N'(a-pyrimidyl)ethylenediamine hydrochloride.

of animals (out of ten) able to tolerate the histamine aerosol for a period greater than twice their individual tolerance (total height of the column), and the number able to tolerate the histamine aerosol for at least ten minutes without dyspnea (shaded part of column). Although greater concentrations than 2 per cent and lesser than 0.25 per cent were used in the case of some drugs the results presented here are confined for the sake of simplicity to the concentrations of 2, 1, 0.5 and 0.25 per cent. In the case of some drugs the aerosols were so irritating, with the production of violent coughing and dyspnea, that the animals were able to tolerate either only a part of the five minutes' exposure or none at all. In any event, the response to the histamine aerosol in these instances must be interpreted in the light of this irritation and the duration of exposure to the drug aerosol. (Table I).

TABLE I. IRRITATION OF SOME ANTIHISTAMINE AEROSOLS IN GUINEA PIGS

	TOLERATION	FIGURES BASED ON
Chlorothen		
2 %—very irritating	2 min. only	2 min.
1 %—very irritating	2 min. only	2 min.
0.5 %—irritating	3 min. only	3 min.
0.25%—OK		
Bromothen		
2 %—very irritating	3 min. only	3 min.
1 %—very irritating	3 min. only	3 min.
0.5 %—slightly irritating	5 min. only	5 min.
0.25%—OK		
Pyrrolazote		
2 %—too irritating	not used	
1 %—too irritating	not used	
0.5 %—irritating	3 min.	3 min.
0.25%—slightly irritating	5 min.	5 min.
3277 RP		
2 %—too irritating	not used	
1 %—very irritating	5 min.	5 min.
0.5 %—very irritating	5 min.	5 min.
0.25%—very irritating	5 min.	5 min.
C-5581 H		
2 %—very irritating	2 min.	2 min.
1 %—irritating	3 min.	3 min.
0.5 %—slightly irritating	5 min.	5 min.
0.25%—slightly irritating	5 min.	5 min.

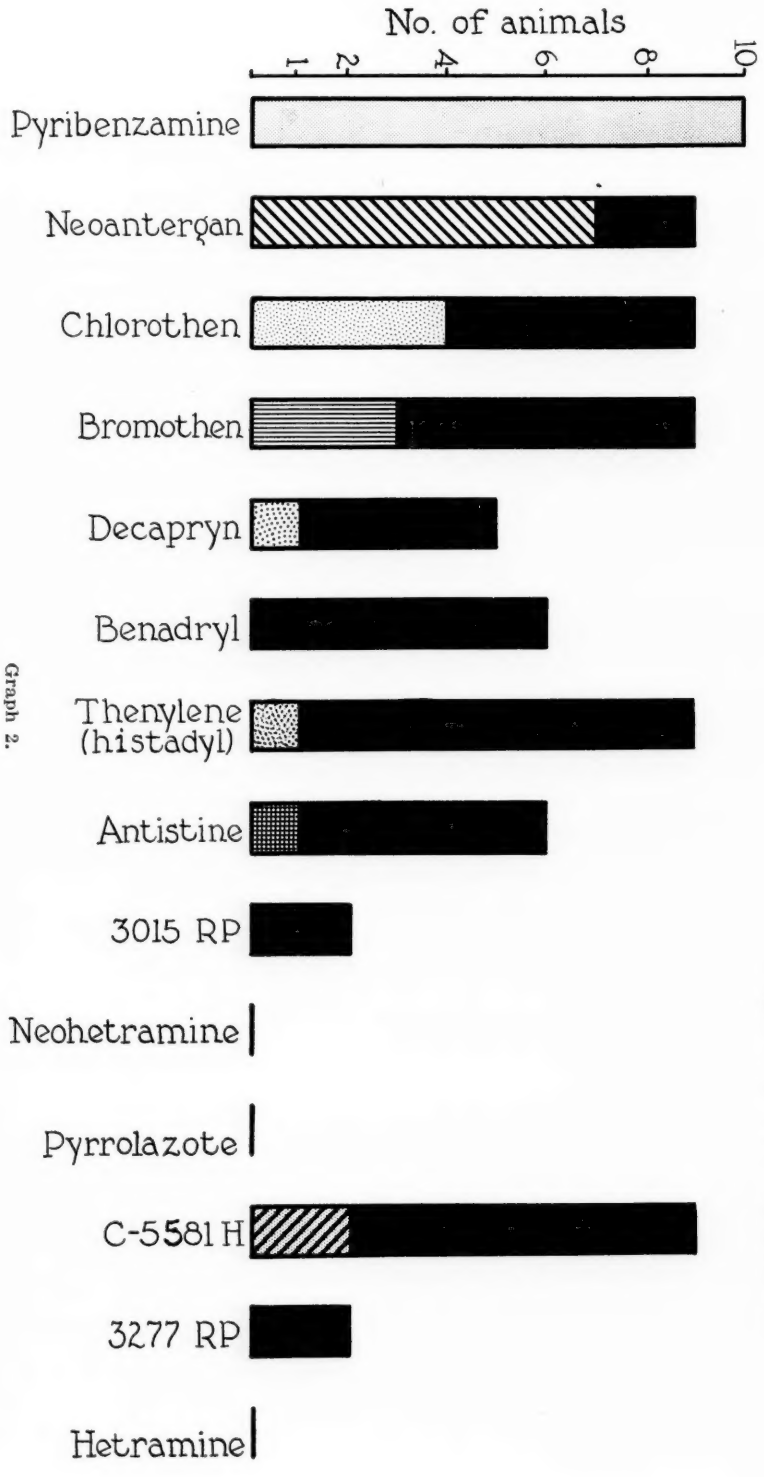
Graphs 1, 2, 3, and 4 illustrate the responses to histamine aerosol after exposure to the antihistamine aerosol in various concentrations. In Graph 1 it is shown that a pyribenzamine aerosol of 2 per cent was able to prevent dyspnea in all of ten animals after a ten-minute exposure to a histamine aerosol. Neoantergan enabled ten animals to tolerate exposure to twice its normal time, while nine of the ten tolerated a 10-minute exposure. Chlorothen and Bromothen did not fare quite as well, but this must be interpreted in light of the fact that because of the irritativeness of these drugs the animals were exposed to these antihistamine aerosols for two and three minutes, respectively, as compared with the standard five-minute period (Table I). The remainder of the fourteen antihistamine drugs are depicted in this graph. Note also that pyrrolazote and 3277 RP were not used because of irritation produced with this concentration and that C-5581 H was used only for two minutes.



Graph 1

2% ANTIHISTAMINE AEROSOLS

Total column = no. of animals tolerating histamine double normal time.
Patterned column = no. of animals tolerating 10 minute exposure to histamine.



Graph 2.

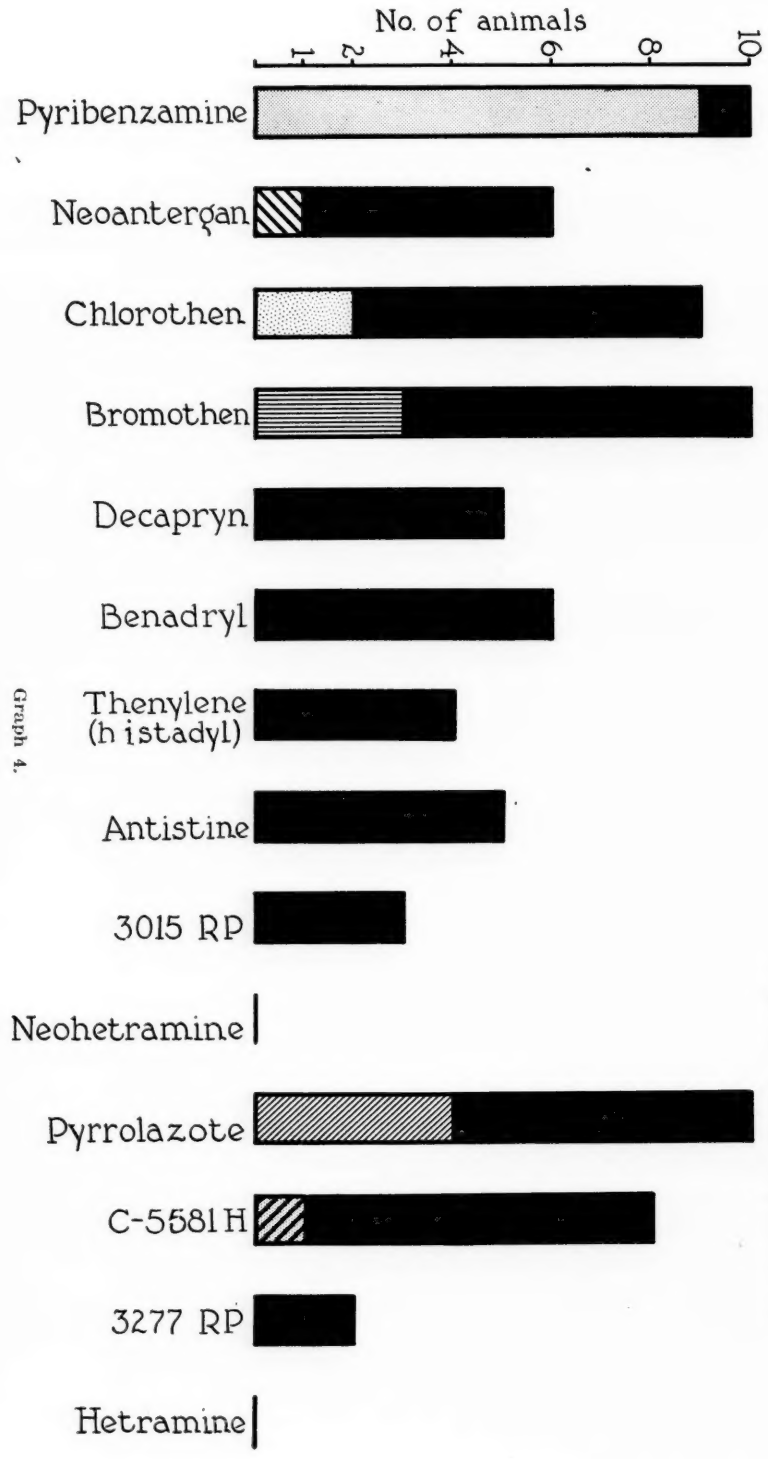
1% ANTIHISTAMINE AEROSOLS

Total column = no. of animals tolerating histamine double normal time.
Patterned column = no. of animals tolerating 10 minute exposure to histamine.

Graph 3.

¼% ANTIHISTAMINE AEROSOLS

Total column = no. of animals tolerating histamine double normal time.
Patterned column = no. of animals tolerating 10 minute exposure to histamine.



Graph 4.

½% ANTIHISTAMINE AEROSOLS

Total column = no. of animals tolerating histamine double normal time.
Patterned column = no. of animals tolerating 10 minute exposure to histamine.

Graph 2 shows the results with 1 per cent antihistamine aerosols, and demonstrates the same general order of effectiveness of the various drugs, with an occasional exception. Part of the exception (as in the case of C-5581 H) is due to the fact that the less concentrated aerosol is less irritating and thus more effective.

Graph 3 shows the results with 0.5 per cent antihistamine aerosols. Here, too, it will be noted that the irritating drugs (Bromothen, Pyrrolazote, 3277 RP, and C-5581 H) may be just as effective or even more effective than in higher concentrations.

Graph 4 illustrates the results with 0.25 per cent antihistamine aerosols. Here the findings become more irregular, and perhaps not as reliable. Nevertheless, since most of the irritating drugs become essentially nonirritating at this concentration, their ability to protect against histamine aerosols in comparison with drugs of known potency (Pyribenzamine and Neoantergan) shows up more favorably than at higher concentrations.

Duration of Action of Aerosols (Table II).—A drug aerosol of a concentration giving the maximum effect for that drug was used. It will be noted that in most instances sixty minutes was the maximum duration of this protection.* This means that the animals were not able to tolerate more than double their normal exposure time to histamine aerosol after this interval. With several of the drugs this duration of action was shorter than sixty minutes, primarily due to the fact that their degree of maximum effectiveness was less marked.

TABLE II. DURATION OF PROTECTION FROM ANTIHISTAMINE AEROSOLS AGAINST HISTAMINE AEROSOLS

AEROSOL	CONCENTRATION	DURATION OF MEASURABLE PROTECTION
Pyribenzamine	2%	60 – 90 min.
	1%	60 min.
Neoantergan	2%	60 min.
Chlorothen	2% (2 min.)	90 min.
Bromothen	2% (3(min.)	90 min.
Decapryn	2%	30 – 45 min.
Benadryl	2%	45 min.
Thenylene (Histadyl)	2%	30 – 45 min.
Antistine	2%	60 – 75 min.
3015 RP	2%	45 – 60 min.
Neohetramine	4%	30 min.
Pyrrolazote	0.5%	60 min.
C-5581 H	1%	60 min.
Hetramine	4%	45 min.

Comparison with Intraperitoneal Injections.—The drug was administered by intraperitoneal injection and its effect noted on the prevention of bronchospasm from aerosolized histamine as in the previous experiments. Both the effectiveness and duration of action were studied. This work has not been completed and we shall report here only on one or two of the results. One milligram of Pyribenzamine per kilogram was the smallest amount required to give

*Mayer and his associates[5] obtained protection against shock from histamine injection for several hours after Pyribenzamine aerosols. However, the long exposure to, and high concentration of the aerosol used make it likely that absorption of the excess explains this difference.

ten-minute protection to all the animals, and the duration of action of this drug was four to six and one-half hours. In the case of Chlorothen, this effect could not be obtained with 1 mg., but could be obtained with 3 mg., with duration of action of four and one-half hours. Histadyl or Thenylene in 3 mg. doses gave protection for three and one-half hours, while Decapryn in 5 mg. doses protected the animals for four hours.

DISCUSSION

Several features of these experiments deserve comment. There is hardly any doubt that it requires less histamine by aerosol than by injection to produce bronchospasm. The following values in our experiments illustrate this point:

Strength of histamine solution = 0.5 mg. (500y) per c.c.
Solution aerosoled per minute = 0.098 c.c. = 49y.
Air flow = 4.0 liters per min.
Amount of histamine per liter = 12y.
Most normal animals had severe dyspnea in less than 3 minutes.
Total histamine aerosoled in chamber in 3 minutes = 147y.
Respiratory ventilation of guinea pig in 3 minutes = 200 to 1,000 c.c.?
Maximum amount histamine a guinea pig can inhale = 12y.

This amount is considerably less than that required to produce histamine shock by injection (30y +).

As regards the comparative effectiveness of aerosol and injection of the antihistamine drug, the following figures are applicable:

Strength of Pyribenzamine giving a maximum protection = 1% for 5 minutes.
Solution aerosoled per minute = 0.095 c.c.
Air flow per minute = 4.7 liters.
Air flow in 5 min. = 23.5 liters or 475y.
Pyribenzamine per liter = 20y.
Maximum amt. inhaled by a guinea pig = 300 to 1,500 c.c. = 30y.

This amount is considerably less (300y.) than that required to produce the same effect by injection.

The above result contributes evidence to the effect that it is possible to prevent asthma, at least in the guinea pig, with much less of the drug applied by aerosol than by injection. This also gives credence to our preliminary findings with antihistamine aerosols in human asthma and furthermore contributes circumstantial evidence to the belief that the failure of the antihistamine drugs in ameliorating asthma when given orally may be due to an insufficient concentration of histamine reaching the bronchi.

There are some valid objections to this technique of aerosols in guinea pigs. There is real difficulty in obtaining an end point; however, with experience this objection may be minimized. Some of the drugs are so irritating by aerosol that an effective concentration cannot be applied. Such drugs could be studied by employing lower concentrations for a longer period of time. We have also begun to make observations on the effect of micronized powders of these drugs

as aerosols. There appears to be a tendency to the production of histamine refractoriness from repeated exposure to histamine aerosols. This requires more extended and exact observations.

SUMMARY

1. Aerosols of antihistamine drugs are effective in the prevention of bronchospasm from histamine aerosols in guinea pigs.

2. The evidence indicates that this preventive effect requires much less of the drug by aerosol than by injection.

3. The duration of action of aerosols is considerably shorter than that by injection, averaging about sixty minutes, as compared to several hours.

4. A comparative study of the findings on fourteen antihistamine drugs is presented. These results indicate that this method is a practical means of assaying the antihistaminic potency of a drug. This does not, of course, mean that the value is comparable necessarily to its antiallergic effectiveness.

5. These findings constitute laboratory evidence for our clinical impression that antihistamine aerosols may be effective in asthma and give us encouragement to proceed with our clinical experimentation.

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Original Articles

MEASUREMENT OF CHANGES IN VITAL CAPACITY AS A MEANS OF DETECTING PULMONARY REACTIONS TO INHALED AEROSOLIZED ALLERGENIC EXTRACTS IN ASTHMATIC SUBJECTS*

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EXPOSURE of asthmatic subjects to dry pollen or to allergenic extracts by inhalation has occasionally been used as a means of testing for specific factors causing asthmatic attacks. The method has the advantage that it is a direct test of the sensitivity of the patient's bronchial tree and lung to the inhaled substances. In a study of fifty-four cases of asthma, Stevens[1] found that inhalation of various allergens caused no symptoms in eleven subjects in whom all skin tests were negative. Among thirty-five subjects showing reactions to one or more extracts by skin test, there were fifteen who suffered asthmatic symptoms following inhalation of certain allergens. No data are presented on individual cases, and it is not possible to determine from this report how well the reactions to inhaled allergens correlated with the clinical sensitivities of the patients. Using inhalation of powdered Alternaria as the method of testing, Harris[2] studied the response of forty-four subjects suspected of mold sensitivity and elicited allergic symptoms, including asthma, in twenty-one. Urbach[3] states that experience with this method of testing has led most workers to the conclusion that asthmatic attacks of considerable severity and duration may be produced, and that it is cumbersome and impractical for diagnostic purposes. Our experience leads us to believe, on the other hand, that valuable information may be obtained by a modification of this type of test both as a diagnostic procedure and as a means of studying the asthmatic attack under controlled conditions.[4]

Using a technique similar to that described by Curry,[5] we have observed that a reduction in vital capacity may follow inhalation of aerosolized pollen extracts. This reduction may be associated with rhinitis, cough, a feeling of tightness in the chest, and wheezing, or the patient may be unaware of any sensation. The method appears to afford a practical means of testing, and one which entails little discomfort to the patient. In most instances, reductions in vital capacity have been moderate in degree and of short duration. In a few instances in which exposure was purposely much more intense than that required to produce an easily measurable reduction in vital capacity, symptoms of hay fever or asthma were marked but never alarming, and they were rarely sufficiently severe as to require administration of adrenalin or other medication.

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METHODS

Asthmatic individuals, presumably pollen sensitive, as judged by history and skin tests, were selected for study. The tests were carried out with the subject rested and comfortably seated. The procedure was explained and a number of preliminary measurements were made in order to acquaint the subjects with the method and to establish a base line on which to judge later measurements. A Parke Davis hand nebulizer was used in some of the earlier tests, but better results were obtained with a No. 40 De Vilbiss nebulizer. Oxygen was delivered to the nebulizer at the rate of 6 liters per minute, controlled by occlusion of the open limb of a glass Y tube. Exposure was carried out by having the patient exhale forcibly and then inhale deeply with the nebulizer held before his open mouth, during which the limb of the Y tube was closed. We are aware of no objection to continuous operation of the nebulizer, except that this entails a waste of extract and concentration by evaporation. There is also the possibility that continuous mixing of the extract with oxygen might impair its activity. At the end of each inspiration, the subject was required to hold his breath for three seconds before expiring in order to bring about better contact of the aerosolized extract with the reacting surface. Intensity of exposure was varied by increasing or decreasing the number of inhalations.

The pollen extracts were prepared in the proportion of 1 Gm. of pollen and 30 ml. of buffered saline solution at pH 7.4 containing 0.5 per cent phenol. A purified house dust extract in a concentration of 2.5 per cent was diluted with extracting fluid to make a final concentration of 0.5 per cent.* Every subject was tested one or more times with the extracting fluid alone. In no instance did this cause a significant reduction in vital capacity or any allergic symptoms. We have no evidence to date, therefore, to indicate, as stated by Urbach,[3] that inclusion of phenol in the extracts administered in this manner is likely to cause symptoms due to irritation.

The vital capacities were measured with a Benedict-Roth Metabolism Machine, and the rate and volume of each expiration were recorded in ink on paper moving at a rate of 21 cm. per minute. Ink recording of vital capacity measurements on a moving drum has a number of advantages. A permanent record is obtained, and notes on the patient's reaction can be written on the drum opposite the vital capacity readings. The rate of expiration tends to be decreased in asthmatic and emphysematous subjects, and we have also seen this occur in a few instances following exposure to aerosolized pollen extracts, even when little change in vital capacity occurred. Only marked changes of this kind would be noticed if simple vital capacity measurements were made, whereas relatively small changes in the rate of expiration are at once apparent as a broadening of the expiratory tracing. Finally, ink recordings help to detect difficulties in the performance of the test due to cough, nervousness, or attempts by the subject to obtain more air while making a tracing.

Measurements of vital capacity were made at one minute intervals or more after exposure to the aerosolized extract. Care was taken to avoid tiring the subject by too frequent measurements. The subjects were questioned as to any subjective sensations, and auscultation of the chest was carried out at intervals.

RESULTS

The results of tests in three patients with asthma are illustrated in Figs. 1 to 3. Fig. 1 shows the changes in vital capacity observed in G.C. following inhalation of extracts of timothy, birch, oak, and ragweed pollen. These tests were done with fifteen inhalations from the small nebulizer, with the exception

*Prepared by Endo Products, Inc., Richmond Hill, N. Y., U. S. A.

of the test with timothy pollen extract which was done with the large nebulizer. The greatest reduction followed inhalation with ragweed. Interference with a full expiration due to cough may have occurred with the lowest reading (four minutes) in the test with this extract, but cough did not interfere with the other readings. Reductions of lesser degree followed inhalation of birch and oak pollen extracts, whereas timothy pollen extract was without effect in spite of the greater exposure administered. These results correlate well with seasonal variations of the patient's symptoms (see legend, Fig. 1). Cough and itching of the throat occurred in each instance in which there was a reduction of vital capacity. Tightness of the chest also accompanied the two greatest reductions in the vital capacity (oak and ragweed) and physical signs of asthma were audible at a time when the vital capacity was less than 70 per cent of the control value. Symptoms of hay fever did not occur. In each instance, the vital capacity returned to the control range within thirty-five minutes.

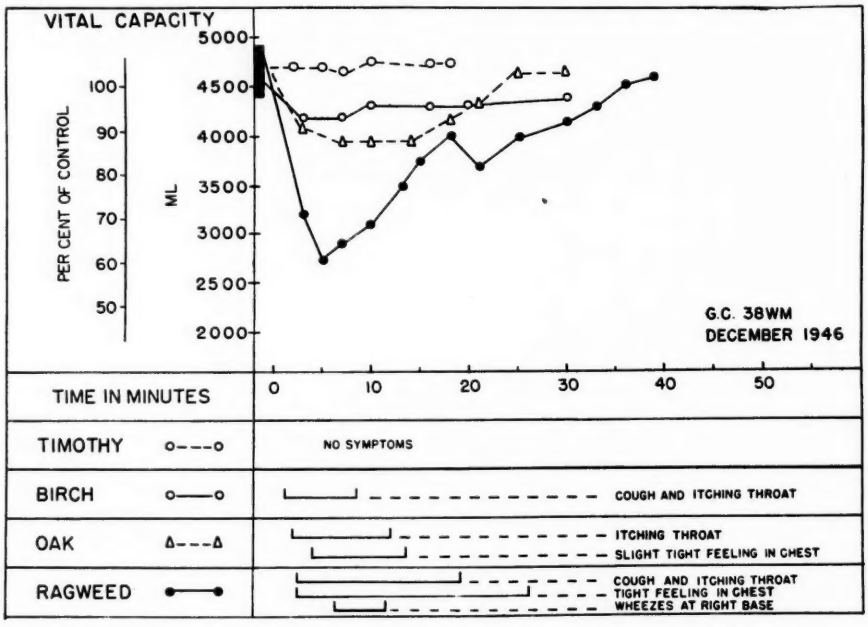

Fig. 1.—Changes in vital capacity following inhalation of aerosolized extracts of pollen. G. C. 38 WM. Hay fever for eleven years, from mid-August to mid-October, associated with asthma, and for the past five years has also had the same symptoms from April to the end of May. Skin tests gave marked reactions to birch, oak, ragweed, and dust. Patient was receiving monthly injections of ragweed extract at time of tests.

In Fig. 2 are shown the results of four tests in a second asthmatic subject, D. D., illustrating a prolonged and marked reduction in vital capacity following exposure to aerosolized extracts of birch and oak pollen. Again, in each instance the reduction in vital capacity was associated with a tight feeling in the chest and, after inhalation of oak extract, there were signs of asthma. The test with oak required only five inhalations with the small nebulizer in order to bring about the reduction in vital capacity shown. That with birch required fifteen inhalations with the small nebulizer. On the other hand, with ragweed and timothy pollen, fifteen inhalations from the large nebulizer failed to cause either a reduction in vital capacity or symptoms. The vital capacity in this patient did not return to the normal range within forty minutes, and in another instance when observation was extended for two hours after exposure to oak, the vital

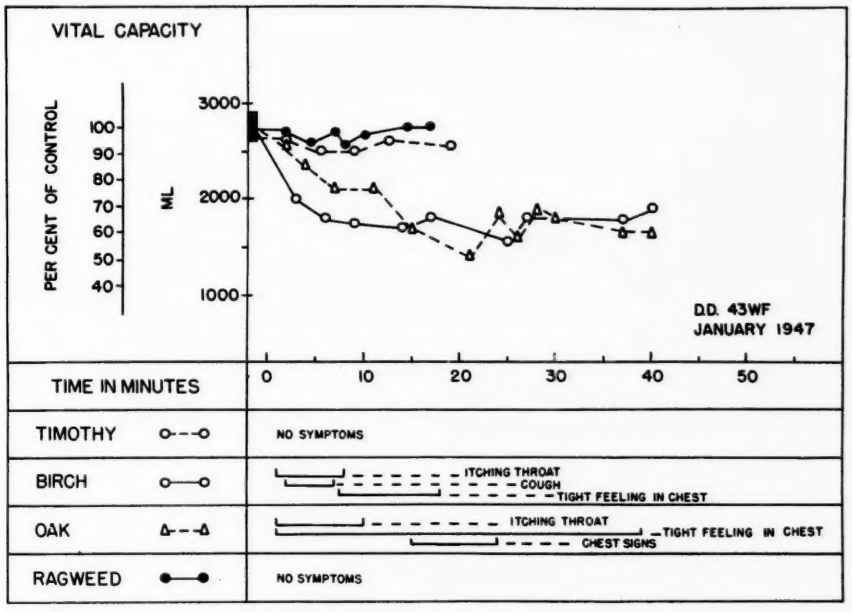

Fig. 2.—Changes in vital capacity following inhalation of aerosolized extracts of pollen. D. D. 43 WF. Hay fever and asthma for years occurring from April to late July. Also has symptoms of hay fever, often with wheezing when exposed to dust or odor from raw celery, parsley or carrots. Asthma was moderately severe. Strong skin reactions to many substances including these mentioned above. Receiving no treatment at time of tests.

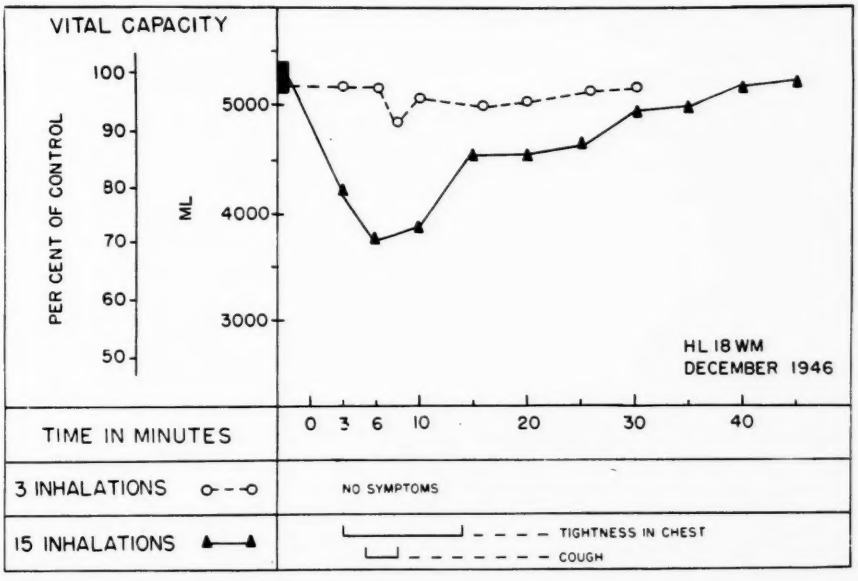

Fig. 3.—Changes in vital capacity following inhalation of aerosolized extract of house dust. H. L. 18 WM. Formerly had skin-sensitivity to ragweed, and hay fever and asthma in August and September. Became entirely symptom-free without treatment and was well until the Spring of 1946 when he developed asthma again which continued irregularly to the present. Repeat skin tests showed a strong reaction to dust and moderate reaction to ragweed and grass pollens. Had asthma for thirty-six hours after an injection of dust extract. Not under treatment at time of tests.

capacity had failed to rise to the normal value. Prolonged reduction in vital capacity was also seen in one other subject, M. W. (Table I). Two tests are shown in Fig. 3 in which an asthmatic subject, H. L., who was clinically sensitive to dust, was exposed first to three and later to fifteen inhalations of dust extract from the small nebulizer. The first exposure caused a small and brief fall in vital capacity, the significance of which is open to question. The second, more intense inhalation carried out thirty minutes later caused a pronounced fall in vital capacity associated with tightness in the chest. Cough, though present, did not interfere with the vital capacity determination.

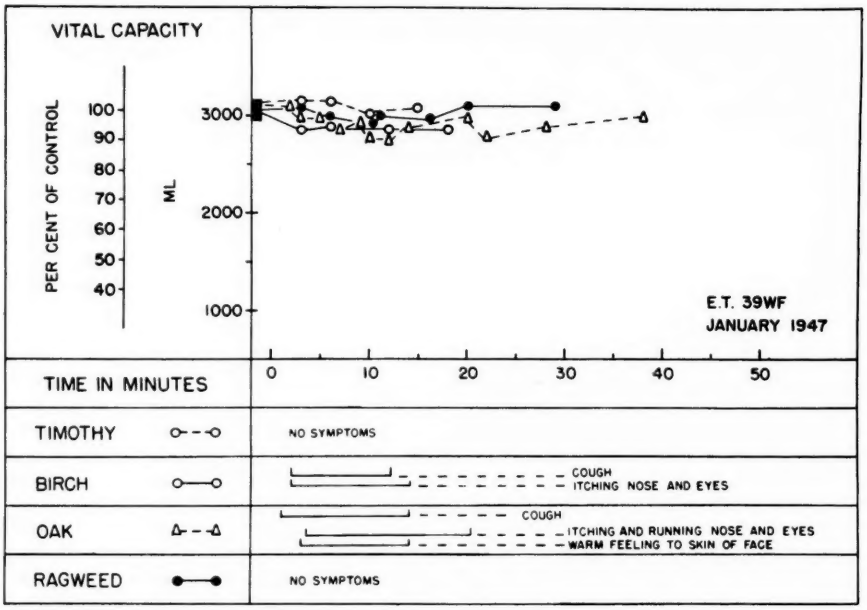

Fig. 4.—Changes in vital capacity following inhalation of aerosolized extracts of pollens. E. T. 39 WF. Hay fever from April to June for ten years. No asthma. Intense skin reactions to birch, oak and poplar pollens and also feathers, dust and cat hair. Treatment with birch and oak pollens was followed by a good clinical result. One severe consitutional reaction following pollen injections. Not under treatment at time of tests.

In Fig. 4 are shown the results of tests with four pollen extracts in a hay fever subject, E. T., who denied having asthmatic symptoms at any time. As can be seen, inhalation of aerosolized extracts of birch and oak produced marked symptoms of hay fever, without producing any reduction in vital capacity. This patient was highly sensitive to birch and oak by skin test, had marked hay fever symptoms from late April to early June, and had suffered one very severe constitutional reaction following an injection of oak extract. Thus, a high degree of allergy to a pollen extract does not necessarily signify that inhalation of the aerosolized allergen will cause a fall in vital capacity nor an asthmatic attack.

The maximum decrease in vital capacity as well as the number of inhalations required to produce this decrease in the three asthmatic subjects mentioned above, as well as in seven additional patients with asthma, are shown in Table I. In all of the subjects, the maximum fall occurred within ten minutes of exposure and in eight of the ten subjects the vital capacity had returned to the control level within one hour. Subject D. D., as mentioned above, and to a lesser degree, subject M. W., showed a more prolonged reduction in vital capacity following inhalation of extracts to which they reacted, and on a number of occasions

TABLE I. EFFECT OF INHALATION OF POLLEN AND DUST EXTRACTS ON THE VITAL CAPACITY OF SUBJECTS WITH ASTHMA

SUBJECT	AGE AND SEX	BIRCH		OAK		TIMOTHY		RAGWEED		DUST		SUBSTANCES KNOWN TO CAUSE ASTHMATIC ATTACKS
		NO. OF INHALATIONS	PER CENT FALL IN V. C.	NO. OF INHALATIONS	PER CENT FALL IN V. C.	NO. OF INHALATIONS	PER CENT FALL IN V. C.	NO. OF INHALATIONS	PER CENT FALL IN V. C.	NO. OF INHALATIONS	PER CENT FALL IN V. C.	
G. C.*	39M	*15*	5	*15*	10	10	0	*15*	35			Spring pollens Ragweed Dust
D. D.*	44F	*10*	40	*5*	42	10	0	10	0			Spring pollens Dust
E. S.	46F	5	0	5	0	5	0	10	28	*15*	22	Ragweed and dust
M. W.	42F	5	50	10	0	10	0	5	21	5	17	Spring pollens Ragweed Dust
M. Mu.	44F	5	0	5	0	5	0	5	0	5	33	Dust
H. M.	37F	10	0	10	57	10	0	10	0			Spring pollens
M. Me.	16F					5	0	5	10	5	0	Dust Ragweed
M. S.	22F					15	0	15	29			Ragweed Dust Many others
H. L.*	19M									*15*	26	Dust ?Ragweed
E. F.	43F							10†	34			Ragweed

Figures in italics represent the number of inhalations made with a Parke Davis nebulizer in place of a No. 40 DeVilbiss nebulizer.

*See Figs. 1, 2, and 3.

†Extract diluted 1:5 for this test. This precaution was taken because this subject had had a severe constitutional reaction following a subcutaneous injection of ragweed pollen extract.

medication was given to restore the vital capacity to the control level before the subject left the laboratory. In no instance did the skin fail to react to the endermal injection of an extract which gave a reduction in vital capacity by our method of testing. However, there were many instances in which substances which reacted by skin test failed to produce any change in the vital capacity when inhaled as an aerosol.

As shown in Table I, the correlation was good between allergenic substances causing reductions in vital capacity on the one hand and clinical sensitivity as judged by statements made by the subject on the other. There is one apparent discrepancy. M. Me., who stated that she was dust sensitive, exhibited no fall in vital capacity following five inhalations of dust extract from the large nebulizer. The possibility exists that more intense exposure in this subject and in others who failed to respond might have produced a fall in vital capacity.

DISCUSSION

Satisfactory performance of the test is dependent on cooperation of the patient. Those individuals who are unable to produce vital capacity readings within 100 or 200 c.c. of their maximum yield tracings which can only be interpreted if reductions in vital capacity are large following inhalation of extracts. Cough, as mentioned earlier, may cause difficulty, occurring most often towards the end of expiration and interrupting it. However, cough, in our experience, has not interfered often, and many very marked reductions in vital capacity were observed during which cough was entirely absent. Our experience to date leads us to believe that cough occurring during these tests is more closely associated with symptoms of the upper respiratory tract, especially those which the patients described as hay fever, and less so with those reactions in the lung which lead to a reduction in vital capacity. Finally, a leak or mechanical difficulty in the apparatus will lower the vital capacity readings and must be guarded against carefully.

The studies in these asthmatic subjects show that, of the criteria used by us for the recognition of pulmonary reactions to inhaled allergenic extracts, the reduction in vital capacity was the most constant. Were it not for the observed changes in vital capacity, the conclusion would have been reached repeatedly that only very slight, and, so far as the lung was concerned, unimportant changes were taking place in the subjects. With one possible exception (Table I, M. Me.), the results to date indicate that reductions in vital capacity following inhalation of extracts of allergens under the conditions of the test occur only in asthmatic individuals in whom such allergens are causative factors in the subject's asthma. However, the test can hardly duplicate what occurs during the pollen season. Pollen is particulate matter and, in addition, may contain active material which is not present in extracts of pollen. Furthermore, during the pollen season, exposure is continuous or, if not continuous, is repeated over many days or weeks. Thus the relative intensity of exposure during the pollen season and in the laboratory may be very different, both with regard to amount and duration. We need hardly expect, therefore, that correlation between symptoms occurring during pollen seasons and sensitivity as indicated by this test will be absolute.

Although this method of testing asthmatic subjects may prove to be valuable in diagnosis, nevertheless it appears probable that the method will have greater value as a means of studying the asthmatic attack under controlled conditions, especially with regard to the influence which various drugs may have in altering the reaction to inhaled pollen extracts. It has been possible, for example, to make a comparison between the effect of various drugs on the pulmonary reaction following administration of histamine and acetyl-beta-methylcholine on the one hand, and the administration of pollen on the other.[6] Such studies may lead to a more precise knowledge concerning the role played by histamine and acetylcholine in the production of the pollen-induced attack. This appears to be feasible today because, in addition to atropine, which is highly specific as an antagonist to acetylcholine, there are also available the antihistaminic drugs which have the single important action of neutralizing the effects of histamine.

SUMMARY

1. It was shown that a reduction in vital capacity followed the inhalation of aerosolized extracts of certain pollens and dust in ten asthmatic subjects.

2. Tests could be carried out with little discomfort to the subject, although mild asthmatic manifestations usually occurred.

3. In some instances a fall in vital capacity was observed in the absence of signs or subjective symptoms of asthma.

4. Our limited experience indicates that this method may be helpful in diagnosis. The technique has the advantage that pulmonary reactions indistinguishable from spontaneous asthma may be produced and measured under controlled conditions.

5. The possible value of this method in studying the effect of drugs on the asthmalike response is pointed out.

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QUANTITATIVE STUDIES IN SKIN TESTING

I(A). The Assay of Ragweed Extracts by Means of Scratch Test Utilizing an "All or None" Response*†

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A NUMBER of different methods have been proposed for the assay, or as it is usually termed, the standardization of allergenic extracts.[1-4] Most of these depend upon the measurement of some physico-chemical property of the extract, e.g., the ratio of the weight of pollen extracted to volume of extracting fluid, the concentration of total nitrogen in the extract, or the concentration of phosphotungstic acid precipitable nitrogen. There has been much controversy as to the relative merits of these various methods of standardization. Indeed, doubt has been expressed as to the relationship, if any, of the values so obtained, to the actual concentration of active principle or principles which cause the various manifestations in the allergic subject.[5]

The logic in these attempts at "chemical standardization" is the following. It is desired to have some relatively simple method which will reliably reflect the biological activity of allergenic extracts. The concentration of active principle or principles determines the degree of reactivity of the extracts. One cannot measure this concentration directly, but it is assumed that the physico-chemical property chosen for measurement is quantitatively correlatable with that of the active material. Therefore, this latter measurement will determine the concentration of active substances and so predict the reactivity of the extract. But the chemical constitution of the active principles is unknown. The exact relationship between active principle and physico-chemical property used for standardization is also unknown.

The problem of standardization thus necessarily becomes the problem of empirically determining the degree of correlation between the chosen physico-chemical property and the biologic activity. At present no valid method of measuring this is available. The degree of correlation is therefore beyond objective determination. Without this objective evidence any discussion of the relative merits of the various methods of standardization is meaningless. Furthermore, the strength of extracts so standardized is characterized only by one quantity, e.g., the milligrams total nitrogen/c.c., or the milligrams phosphotungstic acid precipitable nitrogen/c.c., etc. A given allergenic extract has, however, a variety of biologic activities or functions. It can react directly on the specifically sensitive skin, neutralize reagins, produce and combine with thermostable antibodies, cause constitutional reactions and, it is generally believed,

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yield therapeutic effects in sensitive patients. It is unknown whether a constant quantitative relationship exists among these various functions for extracts of a given allergen, yet unless such a constant relationship exists, no single value could express the strength of such extracts.

The purpose of this paper is to describe a method of measuring one of the biologic functions of ragweed pollen extract, the direct skin reactivity.

PRINCIPLES OF THE ASSAY

Whether one physically measures the mass of a substance by weighing or the concentration of a chemical by means of a color developed in a chemical reaction or the amount of a biologically active substance by means of a biological reaction (biologic assay), the basic principles are the same. The elaborations and complications introduced by a biologic assay are the result of using a biologic system for measurement but do not denote any difference in fundamentals.

If one wishes to assay physically, chemically, or biologically, the amount of a given substance, one measures the change which that substance produces in a system set up so that the change produced is specific for that substance and the amount of change bears some kind of known constant proportionality to the amount of material one desires to measure. The amount of the given substance is then calculated from the amount of change produced in the system by means of the known relationship between the amount of substance introduced and the change yielded in the system.

The change produced in the biologic system (organism, piece of uterus, etc.) used for the measurement or assay of a biologically active substance, is termed the response. The relationship between the amount of change and the amount of active material introduced into the given biologic system is called the dose-response relationship or the dose-response curve. This must be empirically determined. Thus, for a biologic assay one requires a system which will yield a more or less specific response and a knowledge of the dose-response curve.

Broadly, two different types of response are used in biologic assay, the "all or none" or "quantal" response, and the graded or quantitative response. The "all or none" response is an either-or type, death or survival of an animal, presence or absence of convulsions, presence or absence of a skin reaction, etc. The graded response is one that is measurable in terms of some unit, e.g., the height of a blood pressure reading, growth of a root, weight of an organ or animal, etc. The response used in this work was of the "all or none" variety, the presence or absence of a wheal on scratch testing a specifically susceptible individual with a given ragweed extract.

Gaddum[7] and, independently, Bliss,[9] developed (Bliss' terminology) the log-probit dose-response relationship. By means of their general method a great number of different types of substances giving an "all or none" reaction could be assayed and the necessary statistical and mathematical techniques were given a rational theoretical basis. The present work is an application of their

techniques, and for a more comprehensive treatment than the following brief, nonmathematical description, the reader is referred to the articles of Bliss and of Gaddum.[6-8]

A number of different dilutions of a given preparation of the biologically active material to be assayed are prepared. Each dilution is administered to a number of test subjects. The number of positive responses to each concentration is recorded and expressed as the percentage of the total number of subjects to which that dose was administered. This percentage is transformed into so-called probit or probability units. These units are of such a nature that for every percentage there corresponds a probit. Tables have been prepared which give the probits for convenient percentages from 0 to 100 per cent.* The probit found for each concentration is plotted against the logarithms of the concentration. If this method of assay holds for a given substance, the line connecting the points should be straight within the experimental error. The standard preparation against which the activity of the given material is to be compared is tested at the same time in the same way. If this method of assay holds, the two lines obtained should not only be straight, but should be parallel. These are two necessary conditions for a valid assay.

The points where the standard and unknown lines cut the probit 5.000 line are the $E.D._{50}$'s of the two preparations. The $E.D._{50}$, the Effective Dose 50, is that concentration to which 50 per cent of the subjects will just react. On the assumption that if two different preparations give the same response they must contain equal amounts of active material, one postulates that a concentration of the unknown which causes 50 per cent of the test subjects to react contains the same amount of active material as that dose of the standard which also causes 50 per cent of the test subjects to react. Therefore, the ratio of the $E.D._{50}$'s of the standard to the unknown $\times$ 100 gives the per cent strength of the unknown in terms of the standard, or the ratio of potencies of the two preparations.†

Because of the variability of response within and between the groups tested, the postulate of equality of response, meaning equality of concentration, is rarely strictly true. It is the purpose of the elaborate calculations and statistical manipulations described by Bliss and others to make the postulate as true as possible and to set limits within which it has a given likelihood of being true.

GENERAL PLAN OF EXPERIMENT

A stock mixed ragweed extract was carefully diluted with a known amount of buffered saline. The undiluted extract was termed for convenience the "Standard" (S); the diluted extract was called the "Unknown" (U). The strength of the "Unknown" was expressed as the per cent strength of the "Standard." If 1 c.c. of "Standard" was diluted with 1 c.c. of saline, or 0.5 c.c., or 1.5 c.c., the "Unknown" would have a potency of 50 per cent, 66.7 per

*Tables can be obtained from Division of Pharmacology, Food and Drug Administration, Washington, D. C. See also references 6 and 13.

†Actually, the ratio of the potencies is found by algebraically finding the distance between the two lines (standard and unknown), this distance being equal to the logarithm of the ratio of the potencies of the two preparations. One then takes the antilogarithm of this value to find the percentage strength of the unknown. This is mathematically the same process as the above.

cent or 40 per cent, respectively. The same thing was expressed by the statement that the ratio of potencies of the two solutions S and U was 50 per cent, 66.7 per cent, or 40 per cent. The dilution of "Standard" to make the "Unknown" was known only to the technician making up the dilution and was made known to the experimenter only after the assay results had been calculated. The two dilutions were then tested in the manner to be described. It was the object of the experiment to determine objectively the validity of the assay procedure by seeing how close the assayed ratio of the potencies compared with the known "true value."

Known dilutions, usually three in number, of both the "Standard" and the "Unknown" were made. The dilutions or doses were expressed as decimal fractions of the original, e.g., S was diluted 1:3, 1:5, and 1:10, the doses were 0.333, 0.200 and 0.100. The three dilutions of the "Unknown" were made and expressed in the same way.

Three groups of patients were used as assay subjects. The first group, designated as the "Tuesday group" consisted of nine adults, 17 to 55 years of age, of whom two were males. Eight females, 22 to 60 years of age, made up the "Thursday group," while the third, the "Saturday group," were nine children, 6 to 16 years of age, two of whom were females. In each group the individuals were chosen to be as nearly equal in skin sensitivity as possible. This was done by a preliminary testing of all the subjects with the same concentration of ragweed extract and placing those subjects who gave equivalent or nearly equivalent skin tests in the same group. Some patients did not show up for every test period so at times the groups were smaller than indicated.

Each dose was scratch tested on the volar surface of both forearms. The skin test was read at the end of fifteen minutes. The result of the test was designated as positive—the presence of a wheal, or negative—its absence. Controls were run simultaneously on all subjects using physiologic saline. If any patient reacted also to the saline control, that subject was, of course, eliminated.

At times it was found that an assay could not be finished in one session. It was then finished on the same group the following week. This introduced a degree of nonhomogeneity into the data, which tended to increase the error of the assay, but did not affect its validity.

If, as occasionally happened, an individual did not react to any of the doses administered, that subject was ignored in the calculation of the assay.

It was found, as previously demonstrated by Alexander, Bowman, and others,[10, 11] that there was a progressive decrease in sensitivity from forearm to wrist. There was also a difference in sensitivity between ulnar and radial sides of the forearm.

These findings necessitated a somewhat elaborate scheme of mixing up or randomizing the various doses so that the over-all response to any one dose would be solely the effect of the strength of that dose and not reflect the activity of the site on the arm on which the dose happened to be placed. The highest strength of the "Standard" was tested at the same level of the forearm as the "Unknown," and this same pairing was done for the intermediate and lowest doses of "Standard" and "Unknown." The positions of these pairs on the

forearms for any given patient was made a matter of chance by assigning them according to a table of random digits.[12] All doses of "Standard" were placed on the same side of the forearm, either ulnar or radial. If in one patient the "Standard" series was placed on the ulnar side on the right forearm, the "Unknown" series was placed on the ulnar side on the left forearm.* In the next patient the radial and ulner positions for the series was completely reversed and so on.

EXPERIMENTAL RESULTS

Table I gives the data from an actual assay (assay No. 7). In Fig. 1 the probits (column 5, Table I) are plotted against the logarithm of the concentration (column 3, Table I). The two solid lines are the straight lines drawn by eye through the resulting points. The dotted lines are the corresponding lines as calculated by what is essentially the method of "least squares" described by Bliss, and they are the "best fitting" straight lines. The actual details of the calculation of an assay are described fully and simply by Miller, Bliss, and Braun,[6] and the interested reader is referred to their papers. It is these dotted lines which are used to obtain the calculated ratio of potencies and the error of the assay.

TABLE I

16 PATIENTS	DOSE	−LOGARITHM DOSE X	RESULT PER CENT	EMPIRICAL PROBIT
(1)	(2)	(3)	(4)	(5)
	0.143	0.84466	16/32 50.00	5.0000
"Standard"	0.100	1.00000	13/32 40.62	4.7626
	0.050	1.30103	7/32 21.88	4.2237
	0.333	0.47716	18/32 56.25	5.1570
"Unknown"	0.143	0.84466	11/32 31.25	4.5120
	0.100	1.00000	5/32 15.62	3.9898

It has already been pointed out that a necessary condition for a valid assay is that the observed points should differ from the computed straight lines only by chance. Whether this condition is met can be critically and objectively tested by calculating a value known as the χ^2 (Chi square) test for linearity. If χ^2 is less than 3.84, it is generally considered that the points do not deviate from the straight line more than can be accounted for by chance alone. Another condition for a valid assay is that the two lines obtained in an assay should be parallel. This condition was tested by calculating χ^2 for parallelism, or identity of slopes. If this χ^2 is also less than 3.84 one is justified, without any evidence to the contrary, to ascribe any deviation from parallelism to experimental error.

A résumé of the results obtained in the twelve assays which were completed is given in Table II. Three assays were attempted and failed of completion. Two of these incomplete assays were attempted in the beginning of the experiment and it is felt that with greater experience the two could have been completed.

In every one of the twelve completed assays the curves of the standard and unknown were linear and parallel within experimental error as tested by χ^2.

*It is now believed that the same series should be tested on the same side of both forearms, as this makes simpler the comparison of forearms within the same patient.

Thus the two necessary conditions for a valid assay were critically tested and met.

Table II, column 4, gives the average slope of the standard and unknown curves of the assay. The great variation in the slope from assay to assay is the reason why one standard curve cannot be computed and used for all the assays.

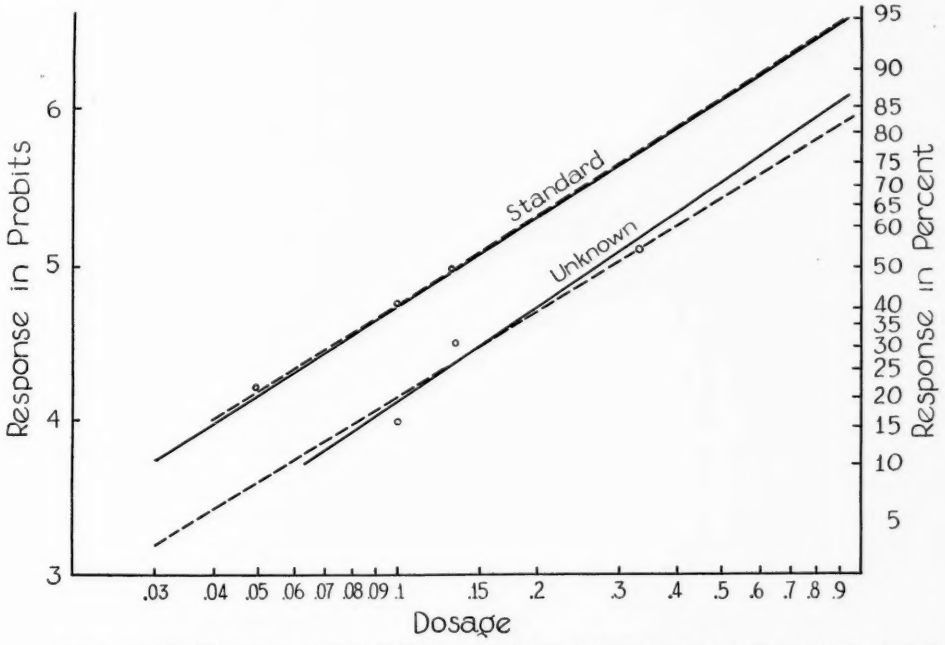

Fig. 1.—Straight line relationship between probit and logarithm of dose for Assay No. 7. ———, Original lines drawn by sight; – – –, lines drawn as calculated mathematically.

The true ratio of potencies (column 5) varied from 75 per cent to 40 per cent. This is a relatively small range but was made necessary by the short working range of the scratch test method, for if too great a dilution is made all reactions will be negative.

Column 6 gives the Calculated ratio of the potencies. The right-hand subcolumn is headed M where $M = \text{logarithm} \frac{\text{Potency of unknown}}{\text{Potency of standard}}$. In the process of calculation of the assay, M, the logarithm of the ratios of the potencies is first determined, and then the antilogarithm of M gives the arithmetic ratio of potencies, m (right-hand subcolumn), which is the value to be compared with the true ratio of potencies (column 5).

An advantage of this method of assay is that it allows one to calculate the error of the assay from the information furnished by the assay. The error is estimated in columns 7, 8, and 9. In calculating the error of the assay, S_M, the logarithmic standard error (column 7) is found. The logarithmic ratio of potencies M (column 6) plus or minus S_M gives the range within which, if the assay were to be repeated a number of times under the same circumstances, approximately two-thirds of the values of M would fall. Thus, the smaller the

TABLE II. ASSAY VALUES CALCULATED MATHEMATICALLY

NUMBER OF ASSAY	DAY	NUMBER OF PATIENTS	MEAN SLOPE OF DOSE RESPONSE CURVES	TRUE RATIO OF POTENCIES (PER CENT)	CALCULATED RATIO OF POTENCIES		LOGARITHMIC STANDARD ERROR S_M	ANTILOG M ± S_M (PER CENT)	CALCULATED AVERAGE STANDARD ERROR (PER CENT)	ACTUAL ERROR (PER CENT)
					ARITHMETIC PER CENT M	LOGARITHMIC M				
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
1	Tues.	1 & 2*	1.6019	75.0	57.1	−0.24342	±0.22012	94.9 - 34.4	±36.4	−17.9
2	Sat.	9	2.9458	75.0	70.4	−0.15251	±0.092092	87.0 - 56.9	±14.9	- 4.6
3	Tues.	9	1.7215	66.7	89.1	−0.050247	±0.15399	137 - 57.9	±28.1	+22.4
4	Tues.	7	1.5389	60.0	30.9	−0.51055	±0.25624	70.1 - 17.1	±18.0	−29.1
5	Tues.	9	0.61773	75.0	115.9	+0.06407	±0.40138	292.0 - 43.9	±107.12	+40.9
6	Tues.	9	2.5414	60.0	37.8	−0.12266	±0.10660	48.3 - 29.6	± 9.28	−22.2
7	Tues	16	1.7664	40.0	47.9	−0.31324	±0.11284	62.1 - 36.9	±12.9	+ 7.9
8	Thur.	10	1.1102	60.0	73.1	−0.13585	±0.26718	135 - 39.5	±35.1	+13.1
9	Tues.	15	1.008	60.0	34.2	−0.46585	±0.25733	60.5 - 15.3	±20.3	−15.8
10	Sat.	17	1.048	58.0	43.6	−0.36070	±0.17170	64.7 - 29.5	±17.2	−14.4
11	Thur.	5	1.5645	40.0	38.0	−0.41976	±0.24071	66.4 - 21.9	±21.1	- 2.0
12	Sat.	7	1.4363	40.0	53.7	−0.27035	±0.20902	86.3 - 33.2	±25.9	+13.7

*Patient tested on two different days.

value of S_M, the smaller the expected range of M and, since m, the ratio of potencies is calculated from M, the more certainly is m, the ratio of potencies, known.

Column 8 gives the range within which the ratio of the potencies, m, would fall in approximately two-thirds of the repetitions of the assay. It should be noted that in every assay but one (assay number 6) the true value for the ratio of potencies was within plus or minus one standard error of the calculated ratio. In this one instance (assay number 6) the true value was within plus or minus two S.E. Thus the calculated error can be used to predict the expected range of actual error.

Because the standard error is calculated as a logarithm when it is translated into arithmetic units as in column 8 the range is not symmetrical. If S_M is relatively small, this asymmetry may not be great, and an approximate arithmetic S.E. may be convenient. This is obtained from Cochrane's formula as given by Miller, Bliss, and Braun.[6] The results of this calculation are given in column 9.

Column 10 gives the actual error for each assay. This varied from +40.9 per cent to –29.1 per cent. The average actual error of these twelve assays was –0.588 per cent. The standard error was ±6.326. Fisher's "t" test,[14] though unnecessary in this instance, indicates the difference of –0.588 per cent from 0 per cent, which is the theoretical expected average actual error, is not significant. This demonstrates that the calculated ratio of potencies for the data neither under- nor overestimates the true ratio of potencies. Thus there is evidence that an unbiased estimate of the true ratio of potencies is obtained by this assay method.

DISCUSSION

Many of the criticisms leveled at the use of the direct skin reaction as a measure of activity of allergenic extracts are an expression of an inability to take into account the great inherent variability within and between patients and an implicit belief that the response must be a linear function of dose.[15, 16] This variability of test subjects is a problem with which biologists in many fields have struggled and the solution described here, the use of a number of test subjects and randomizing of the placing of doses within the patient, and the statistical calculations of results, has been the tool providing the solution. It is because of this variation of test subjects and the unavoidable differences in technic from test to test that the rather elaborate design and calculations are required.

It is also clear that in this instance the response (per cent of positive responses) is not linearly related to concentration, i.e., as one increases the concentration by a certain amount, the per cent of positive responses does not increase by a constant amount. If one finds a concentration which will give a certain percentage of positive responses e.g., 30 per cent positive responses, if another preparation tested at the same time gives 60 per cent positive responses, one cannot conclude the concentration of active material of the second preparation is twice the concentration of the first.

It is probable that some sort of nonlinear relationship holds for dose and response in the testing of allergenic extracts no matter how the response is

measured; whether quantitatively, by actual measurement of size of wheal or erythema, etc., or semi-quantitatively in terms of arbitrary symbols such as 0, +, ++, +++, etc. Thus, no matter what the response chosen one must empirically determine the quantitative relationship between dose and response before one can use the response to tell anything of the concentration of active material.

The log probit relationship has been shown by the data of Table II, to hold for the solutions of ragweed pollen extract tested. The linearity of the curves obtained and the parallelism between the curves provide the necessary conditions for a valid assay. Since the lowest dose tested in a given assay was about 1/20 to 1/50 of the highest, this linearity extends practically throughout the working range of the scratch test. By comparing solutions of known strength it has been possible to demonstrate that the assay results agree within the calculated experimental error with the actual known results. The evidence is clear that not only a valid comparison of two solutions can be obtained but the calculated error is a good estimate of the actual error.

There are a number of factors which affect the error. These are clearly and ably discussed by Miller and associates.[6] One of the most important is the slope of the dose response curves. The slope in this assay was found to be relatively small (Table II, column 4). This means the error will be correspondingly large. This is partially overcome by the experimental design whereby all doses are given in duplicate to all patients. This means that the 16 patients in assay number 7, all other things being equal, are equivalent to 192 test subjects (16 patients $\times$ 2 arms $\times$ 6 doses) in the usual assay where only one dose is given to each subject.

The slope in the usual type of assay reflects the variability of members of the test group. In this type of assay (all doses given to each subject) the slope reflects the variability within and between subjects. In Assay number 1 (Table II) one subject was used twice, yet the mean slope 1.6019 was of the same order of magnitude as in other assays where a number of patients were used. This suggests that the smallness of the slope and thus the largeness of the error is probably determined in large part by the variability within the individual patient.

Having demonstrated that the probit log dose curve is linear, it is only necessary to use two doses of the "standard" and two doses of the "unknown" in future work. This would simplify the calculations especially if the interval between the two doses of standard would be made equal to the interval between the two doses of the unknown.

The calculations are long, cumbersome, and time-consuming in the extreme. A description of a much shorter, simpler graphic procedure will be the subject of another paper. A defect of this assay is inherent in the very fact of skin testing itself. Crude extracts probably contain a number of different skin reactive substances.[17] If this is true and they differ markedly in the range and strength of their reactivity the skin reaction is a mean of the reactibility of these various fractions and the assay value is also an average potency. Thus, two extracts may differ widely in the quantitative spectrum of active principles, but the average potency found on assay may be the same. This is, as pointed

out, inherent in the use of the skin test response. However, if the individuals tested have a different sensitivity to the individual fractions, a significant difference in slope might be found in testing two extracts with differing amounts of individual active principles.

This assay procedure measures only the direct skin reactivity of allergenic extracts. It has been shown to be valid only for scratch tests; however, its application to endermal testing will be reported later. Other measures of the activity of extracts, passive transfer tests, neutralizing ability, ability to react with thermostable antibody, etc., will have to be worked out in turn.

SUMMARY

1. The log probit method of Bliss has been demonstrated to be a valid method of assaying the average direct skin reactivity of ragweed pollen extracts by means of the scratch test. This method not only gives a valid estimate of the comparative strength of such extracts but a valid estimate of the error of the assay.

2. The assay procedure holds only under well-defined conditions. A number of subjects must be tested under as nearly identical conditions of technique as is possible. The assay subjects should be nearly identical in their degree of skin sensitivity as is practical. The placing of the test doses on the arm must be random. For strict comparative measurements a standard should be run with the unknown solutions.

My sincere appreciation is expressed to Miss L. Byrne for her care in making the numerous dilutions required and to members of the Allergy Unit and Department of Biological Chemistry of the University of Illinois College of Medicine for their advice and encouragement.

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QUANTITATIVE STUDIES IN SKIN TESTING

I(B). The Graphic Solution of the Assay of Ragweed Extracts by Means of Scratch Test Utilizing an "All or None" Response

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IN THE first paper of this series[1] a method of assay of the direct skin reactivity of ragweed pollen extract was described. This was shown to be a valid method of assay, and the error of the assay calculated by this method was shown to be a valid estimate of the actual error of the assay. Though the results obtained from this method were shown to be satisfactory, the calculations involved are cumbersome and time-consuming in the extreme. A graphic solution which, while involving a number of approximations and thus not expected to be as accurate, but requiring only a minimum of calculations, will now be described. This is an extension of the method of Miller and Tainter.[2] The data of this paper are the same as used in the preceding paper. These data were independently recalculated by the graphic method to be described and the assay values so obtained were compared with the known "true value" and the assay values obtained by the longer, more trustworthy "mathematical" calculations of the preceding paper.

In this method, the per cent of positive responses are obtained exactly as described in the preceding paper.[1] From these per cents the probits are obtained. The probits are plotted against the dose on log probit graph paper,[2] and a straight line is fitted to these points by eye (e.g., black line in Fig. 1 in preceding paper[1]). Up to this point the procedure is the same as the first part of the previously described method. In drawing these lines one should remember that the points around probit 5.000 are given the most weight and the line should be drawn so as to fall nearer these points.

Instead of carrying out the calculations as in the previous paper,[1] the following procedure was used. Where the straight lines, for "Standard" and "Unknown" across the probit 5.000 line the doses for both standard and unknown are read off. These concentrations are the $E.D._{50}$'s of the "Standard" and "Unknown," i.e., the dose to which 50 per cent of the individuals will react. On the assumption that the solutions are qualitatively identical, the $E.D._{50}$'s are a measure of the activity of the two solutions (compare reference 1). Therefore, the ratio of the two $E.D._{50}$'s ("Standard" and "Unknown") is the ratio of the potencies of the two solutions. Miller and Tainter also give a method for graphically finding the standard error (S.E.) of this $E.D._{50}$. The approximate standard error of the $E.D._{50}$ is given by the formula:

$$\text{S.E. of } E.D._{50} = \frac{2s}{\sqrt{2N'}}$$

In this formula 2s is the value obtained by subtracting the dosage corresponding to probit 4.000 from the dosage corresponding to probit 6.000. The dosages

are read off the graph. "N' . . . is the total number of subjects in the groups which, from the best fitting line would be expected to show effects between . . . probits 3.50 and 6.50."[2]

The standard error of the ratio of the potencies, the error of the assay, is obtained from the formula for the standard error of a quotient.[3]

$$\text{S.E. of "S"/"U"} = \text{"S"/"U"} \sqrt{\frac{(s^2)_s}{S^2} + \frac{(s^2)_u}{U^2}}$$

$$S/U = \frac{\text{E.D.}_{50} \text{ of standard solution}}{\text{E.D.}_{50} \text{ of unknown solution}} \times 100$$

S/U = ratio of potencies
$(s^2)_s$ = squared standard error of E.D.$_{50}$ of standard solution
$(s^2)_u$ = squared standard error of E.D.$_{50}$ of unknown solution

The full calculations of the graphic solution of an assay, assay No. 7 follows. The original data and the graph are taken from the previous paper[1] (Fig. 1 and Table I).

Standard

E.D.$_{50}$ = 0.143
Probit 4.0 = 0.0370
Probit 6.0 = 0.543
$2s = 0.543 - 0.0370 = 0.506$

$$\text{S.E. of E.D.}_{50} = \frac{2s}{\sqrt{2N'}} = \frac{0.506}{\sqrt{2 \times 3 \times 32}} = \pm 0.038$$

Unknown

E.D.$_{50}$ = 0.270
Probit 4.0 = 0.0858
Probit 6.0 = 0.846
$2s = 0.846 - 0.0858 = 0.7602$

$$\text{S.E. of E.D.}_{50} = \frac{0.7602}{\sqrt{2 \times 3 \times 32}} = \pm 0.0548$$

$$\text{The ratio of the potencies, } m' = \frac{\text{"S"E.D.}_{50}}{\text{"U"E.D.}_{50}} = \frac{0.143}{0.270} = 53.0 \text{ per cent}$$

This is to be compared with the value obtained by the longer and more exact method of calculation, 47.9 per cent and the "True value" of 40.0 per cent.

The error of this ratio of the potencies is:

$$\text{S.E.} = S/U \sqrt{\frac{(\text{S.E.})_s^2}{S^2} + \frac{(\text{S.E.})_1^2}{U^2}} = \frac{0.143}{0.270} \sqrt{\frac{(0.0380)^2}{(0.143)^2} + \frac{(0.0548)^2}{(0.270)^2}}$$

$$= 17.7 \text{ per cent}$$

The ratio of the potencies ± its standard error, by this short graphical method is 53.0 per cent ± 17.7 per cent. This approximation agrees reasonably well with the calculated value 47.9 per cent ± 12.9 per cent (Table II in reference 1).

Table I gives the assay values calculated by this graphic procedure for all twelve assays. The values for m', the graphic ratio of potencies, should be compared with m, calculated ratio of potencies. The mean actual error of the "assay calculated graphically" is +2.41 ± 6.29. The application of Fisher's "t" test[4] demonstrates that this error does not differ significantly from the theoretically expected mean actual error of 0 per cent. Thus, for this data, the graphic solution neither under- nor overestimates the true value. The

average difference between calculated and graphic ratio is –4.35 ± 2.17. Applying Fisher's "t" test[4] shows this difference is not significant at the 5 per cent level. However, the p value is sufficiently close to 0.05 to suggest that further data might demonstrate that the graphically calculated ratio tends, on the average, to overestimate slightly the more accurate "maximum likelihood" solution.

TABLE I. ASSAY VALUES CALCULATED GRAPHICALLY

NUMBER OF ASSAY	$E.D._{50} \pm$ S.E. "STANDARD"	"UNKNOWN"	TRUE RATIO OF POTENCIES (PER CENT)	"GRAPHICALLY" CALCULATED RATIO OF POTENCIES (PER CENT)	"GRAPHICALLY" CALCULATED STANDARD ERROR (PER CENT)	ACTUAL ERROR (PER CENT)
(1)	(2)	(3)	(4)	(5)	(6)	(7)
1	0.0985 ± 0.0303	0.140 ± 0.0774	75.0	70.4	±23.7	- 4.6
2	0.135 ± 0.0271	0.280 ± 0.0309	75.0	64.9	±12.0	-10.1
3	0.320 ± 0.0921	0.405 ± 0.100	66.7	79.3	±28.1	+12.6
4	0.100 ± 0.0291	0.235 ± 0.0442	60.0	42.6	±14.7	-17.4
5	0.140 ± 0.105	0.104 ± 0.110	75.0	133	±137	+58.0
6	0.137 ± 0.0314	0.335 ± 0.0612	60.0	41.9	±12.0	-18.1
7	0.143 ± 0.0380	0.270 ± 0.0548	40.0	53.0	±17.7	+13.0
8	0.470 ± 0.0495	0.630 ± 0.0655	60.0	74.6	±11.3	+14.6
9	0.128 ± 0.0733	0.390 ± 0.0893	60.0	35.1	±29.9	-24.9
10	0.120 ± 0.0526	0.270 ± 0.113	58.0	44.0	±19.1	-16.0
11	0.109 ± 0.0419	0.308 ± 0.161	40.0	35.5	±23.1	- 4.5
12	0.135 ± 0.0857	0.253 ± 0.0893	40.0	53.4	±28.2	+13.4

In all but three assays the actual error is less than one S.E. and in these three the actual error is less than two S.E.'s. The average difference between the calculated average S.E. and graphic S.E. is –0.531 ± 3.58, which is far from significant showing that the S.E. calculated in the two different ways do not differ from each other in any consistent manner.

DISCUSSION

A graphic method of calculating the assay of ragweed pollen extracts has been described. It has been proposed because of the extremely lengthy computations required by the first described method.[1] It must be stressed that this shorter method is a very approximate one. In this graphic solution, neither the linearity of the dose-response curves nor their parallelism are tested. Yet these are as necessary conditions for the second method of calculation as for the first.[1] The data of the two curves, "Standard" and "Unknown," are not combined to obtain the ratio of potencies. This means the ratio of the potencies is less securely determined by the second method than the first. The formulae used in calculating the standard error of the ratio of the potencies are themselves approximations. Yet with all these approximations the data of Table I demonstrate that this simple graphic solution can give a reasonably good estimate of the true value and of the error of this estimate. The great advantage of this latter method is that ten minutes or less are required to calculate the results of an assay by the graphic method while the first described method takes two to four hours. In all doubtful cases, e.g., where linearity or parallelism are in doubt, or in critical cases, the longer more accurate method should be used.

SUMMARY

1. A short graphic method of calculating the assay of ragweed pollen extracts is described. The $E.D._{50}$ and the standard error of the two solutions to be compared are found by the method of Miller and Tainter. The ratio of the $E.D._{50}$'s so found is taken as the ratio of the potencies of the two solutions, and using the formula for the standard error of a quotient one then obtains the standard error of the ratio of the potencies.

2. The validity of this procedure, notwithstanding the number of approximations involved, is demonstrated by the data provided. The nature and number of approximations involved are stressed.

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REACTIONS TO ANTIPNEUMOCOCCAL RABBIT SERUM*†

VIII. Effect of Varying Shocking Doses of Antiserum on Reversed Passive Anaphylaxis when Sensitizing Dose of Polysaccharide and Interval Between Sensitizing and Shocking Doses are Constant

IX. Comparison of Reversed Passive Anaphylactogenic Properties of Samples of Antipneumococcal Rabbit Serum, Types II, VII, and VIII.

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PROMPT anaphylactic reactions to therapeutic injections of antipneumococcal serum are common. Conventional anaphylaxis is the accepted mechanism for the reactions which occur when skin tests for sensitivity to serum are positive. However, tests for sensitivity were negative in nearly 80 per cent of a large series of reactions to antipneumococcal horse serum.[1] Evidence has been offered that reversed passive anaphylaxis mediates reactions to antiserum which occur when tests for sensitivity are negative.[2]

These are two of a series of experiments[3-5] still in progress designed to clarify some of the problems of reactions to therapeutic antisera.

Experiment VIII (see title).—Guinea pigs weighing from 325 to 640 Gm. were injected intravenously with constant sensitizing doses of 1.0 mg. of type VIII pneumococcal polysaccharide. After an interval of about sixty minutes, they were given shocking doses of type VIII antipneumococcal rabbit serum intravenously. Lot No. 418, containing 3400 units per milliliter was used throughout. These doses were varied from 3.0 to 0.01 milliliter.

Preliminary experiments had shown that reversed passive anaphylaxis could be produced regularly with a sensitizing dose of 1.0 mg. of polysaccharide and a shocking dose of 2.0 ml. of lot 418 antiserum.

The five negative reactions to 0.03 and 0.01 ml. of antiserum and the failure of three animals to react to 3.0 ml. when they had not had polysaccharide served as controls. There were no reactions to the sensitizing doses of 1.0 mg. of polysaccharide.

Table I shows that fatal or severe anaphylactic shock followed all doses between 3.0 ml. and 0.3 ml. of the antiserum. Shocking doses between 0.2 ml. and 0.05 ml. caused mild reactions regularly. Only one dose below 0.05 ml. caused any detectable reaction.

Experiment IX (see title).—Marked variation in reversed passive anaphylactogenic properties of different lots of antiserum was responsible for considerable difficulty in completing experiment VIII. Five samples from three types of antisera were studied before a suitable lot was found. Variations in different lots of type I antipneumococcal rabbit serum have been described.[5]

Table II gives in detail some differences encountered with two lots of type II, one lot of type VII and one lot of type VIII serum. These results are in marked contrast to the uniformly severe reactions to lot No. 418, type VIII, antiserum shown in Table I.

*From the Allergy and Arthritis Division, Department of Internal Medicine, University of Virginia Medical School, Charlottesville, Va.

†This study was made possible by generous supplies of antipneumococcal rabbit serum by Wyeth, Inc., and of pneumococcal polysaccharide by Lederle & Co.

TABLE I. EFFECT OF VARYING SHOCKING DOSES OF ANTISERUM* ON REVERSED PASSIVE ANAPHYLAXIS, WHEN SENSITIZING DOSE OF POLYSACCHARIDE† AND INTERVAL BETWEEN SENSITIZING AND SHOCKING DOSES ARE CONSTANT

GUINEA PIG	SENSITIZING DOSE	INTERVAL	SHOCKING DOSE	RESPONSE
1	1.0 mg.	±60 min.	3.0 ml.	AD
2	1.0 mg.	±60 min.	2.0 ml.	AD
3	1.0 mg.	±60 min.	2.0 ml.	AD
4	1.0 mg.	±60 min.	1.5 ml.	AD
5	10. mg.	±60 min.	1.0 ml.	AD‡
6	1.0 mg.	±60 min.	1.0 ml.	++
7	1.0 mg.	±60 min.	0.5 ml.	AD
8	1.0 mg.	±60 min.	0.5 ml.	AD
9	1.0 mg.	±60 min.	0.5 ml.	AD
10	1.0 mg.	±60 min.	0.3 ml.	AD
11	1.0 mg.	±60 min.	0.3 ml.	AD
12	1.0 mg.	±60 min.	0.3 ml.	++
13	1.0 mg.	±60 min.	0.2 ml.	+
14	1.0 mg.	±60 min.	0.2 ml.	+
15	1.0 mg.	±60 min.	0.2 ml.	+
16	1.0 mg.	±60 min.	0.1 ml.	++
17	10. mg.	±60 min.	0.1 ml.	+
18	1.0 mg.	±60 min.	0.1 ml.	+
19	1.0 mg.	±60 min.	0.05 ml.	+
20	1.0 mg.	±60 min.	0.05 ml.	+
21	1.0 mg.	±60 min.	0.05 ml.	+
22	1.0 mg.	±60 min.	0.03 ml.	+
23	1.0 mg.	±60 min.	0.03 ml.	0
24	1.0 mg.	±60 min.	0.03 ml.	0
25	1.0 mg.	±60 min.	0.01 ml.	0
26	1.0 mg.	±60 min.	0.01 ml.	0
27	1.0 mg.	±60 min.	0.01 ml.	0

AD = Anaphylactic death. Necropsy = pale, ballooned lungs, scattered hemorrhagic areas, bronchial exudate.

AD‡ = Marked immediate reaction, died less than eight hours.

++ = Moderate to marked reaction = quite sick, convulsive movements, marked dyspnea.

+ = Slight anaphylactic reaction = ruffled hair, sick, expiratory dypsnea.

0 = No reaction.

*Shocking antiserum = Type VIII antipneumococcal rabbit serum, lot No. 418.

†Sensitizing Antigen = 1.0 mg. type VIII pneumococcal polysaccharide.

DISCUSSION

Quantitative roles of antigen[3] and of antibody (Table I) in the production of reversed passive anaphylaxis have been established in principle. The interval between sensitizing and shocking doses seems unimportant so long as circulating antigen persists in significant quantity.[5]

Data accumulated in this series of experiments[2-5] to date would seem to justify the following precautions: One should determine the presence or absence of circulating polysaccharide[4] before giving antiserum to a pneumonia patient. If circulating polysaccharide is present, even when tests for sensitivity are negative, serum should be given with great caution. A safe test dose has not been determined. Doses as small as 0.3 ml. may be fatal to guinea pigs. Marked difficulty occurred in one animal receiving only 0.1 ml. (Table I).

Experiment IX, Table II, emphasizes the marked variation in reversed passive anaphylactogenic properties of different lots of antiserum irrespective of type. No hint of the explanation of these variations is available yet. The solution of this problem would seem to be important to a better understanding of these reactions to antipneumococcal rabbit serum.

TABLE II. COMPARISON OF REVERSED PASSIVE ANAPHYLACTOGENIC PROPERTIES OF SAMPLES OF ANTIPNEUMOCOCCAL RABBIT SERA, TYPES II, VII AND VIII

GUINEA PIG	SENSITIZING DOSE POLYSACCHARIDE	INTERVAL	SHOCKING DOSE ANTISERUM	REACTION
		Type II, Lot No. 42701		
1	1.0 mg.	±60 min.	3.0 c.c.	++
2	1.0 mg.	±60 min.	3.0 c.c.	++
3	1.0 mg.	±60 min.	2.0 c.c.	++
4	1.0 mg.	±60 min.	2.0 c.c.	++
		Type II, Lot No. A.421.1		
1	1.0 mg.	±60 min.	2.0 c.c.	+
2	1.0 mg.	±60 min.	2.0 c.c.	+
3	1.0 mg.	±60 min.	1.0 c.c.	++
		Type VII, Lot No. 220		
1	1.0 mg.	±60 min.	2.0 c.c.	AD
2	1.0 mg.	±60 min.	2.0 c.c.	AD
3	1.0 mg.	±60 min.	2.0 c.c.	++
4	1.0 mg.	±60 min.	2.0 c.c.	+
5	1.0 mg.	±60 min.	2.0 c.c.	+
6	1.0 mg.	±60 min.	2.0 c.c.	+
7	10. mg.	±60 min.	1.5 c.c.	+
8	1.0 mg.	±60 min.	1.5 c.c.	+
9	1.0 mg.	±60 min.	1.0 c.c.	AD
10	1.0 mg.	±60 min.	1.0 c.c.	+
11	1.0 mg.	±60 min.	1.0 c.c.	+
12	1.0 mg.	±60 min.	1.0 c.c.	+
13	1.0 mg.	±60 min.	1.0 c.c.	+
14	1.0 mg.	±60 min.	1.0 c.c.	+
15	1.0 mg.	±60 min.	1.0 c.c.	+
16	1.0 mg.	±60 min.	1.0 c.c.	0
		Type VIII, Lot No. 42901		
1	1.0 mg.	±60 min.	3.0 c.c.	+
2	1.0 mg.	±60 min.	2.0 c.c.	+
3	1.0 mg.	±60 min.	2.0 c.c.	+
4	1.0 mg.	±60 min.	2.0 c.c.	+
5	1.0 mg.	±60 min.	2.0 c.c.	+
6	1.0 mg.	±60 min.	2.0 c.c.	+

AD = Anaphylactic death. Necropsy = pale ballooned lungs, scattered hemorrhagic areas, bronchial exudate.

++ = Moderate to marked reaction = quite sick, convulsive movements, marked dyspnea.

+ = Slight anaphylactic reaction = ruffled hair, sick, expiratory dyspnea.

0 = No reaction.

SUMMARY

Additional studies of reactions to antipneumococcal rabbit serum are presented which show:

1. Shocking doses of antiserum can be reduced to an ineffectual level when sensitizing doses of polysaccharide and the interval between sensitizing and shocking doses are constant. The intensity of the reactions decreased (as antiserum doses were reduced) from uniformly severe to uniformly mild reactions before ineffectual doses were reached.

2. Different lots of the same type and of different types of antipneumococcal rabbit serum vary widely in their effectiveness in producing reversed passive anaphylaxis.

3. Reasons are given for the suggestion that the presence or absence of circulating polysaccharide should be determined before giving antipneumococcal rabbit serum.

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REACTIONS TO ANTIPNEUMOCOCCAL RABBIT SERUM*†

X. Effect on Reversed Passive Anaphylaxis of Varying Speed of Injections of Constant Shocking Doses of Antipneumococcal Rabbit Serum

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PROMPT anaphylactic reactions to therapeutic injections of antipneumococcal rabbit serum are common. Conventional anaphylaxis is the accepted mechanism for the reactions which occur when skin tests for sensitivity to serum are positive. However, tests for sensitivity were negative in nearly 80 per cent of a large series of reactions to antipneumococcal horse serum.[1] Evidence has been offered that reversed passive anaphylaxis mediates reactions to antiserum which occur when tests for sensitivity are negative.[2]

This is one of a series of experiments,[3-5] still in progress designed to clarify some of the problems of reactions to theapeutic antisera.

Experiment X was designed to determine the effect on reversed passive anaphylaxis of varying the speed of injection of constant shocking doses of antipneumococcal rabbit serum. Sensitizing doses of type specific polysaccharide and the interval between sensitizing and shocking doses were constant.

Medium sized guinea pigs were sensitized with intravenous injections of 1.0 mg. of type 8 pneumococcal polysaccharide. One hour later they were given shocking doses of 0.5 ml. of type 8 antipneumococcal rabbit serum, lot 418, 3,400 units ml. It had been shown earlier that 0.5 ml. was a uniformly effective dose. The speed of injection of shocking doses was varied. In different groups of pigs the injection time was fifteen, thirty, sixty, ninety, one hundred and eighty, and three hundred seconds, respectively.

In the first two groups the shocking dose volume was 0.5 ml. An equal volume of saline was added in the last four groups to simplify the injection of the small amount over the longer time. No effect of this dilution to 1.0 ml. was noted.

The results are shown in Table I. It is obvious that all injections which took less than sixty seconds, and most of those which took ninety seconds, were followed by severe anaphylactic reactions. In sharp contrast are the mild or negative reactions when the injection times were one hundred and eighty and three hundred seconds, respectively.

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†This experiment was made possible by generous supplies of antipneumococcal rabbit serum by Wyeth Incorporated and of pneumococcal polysaccharide by Lederle Laboratories, Incorporated.

TABLE I. REVERSED PASSIVE ANAPHYLAXIS. EFFECT OF VARYING SPEED OF INJECTIONS OF CONSTANT SHOCKING DOSE (0.5 ML. = 1,700 UNITS) OF TYPE 8 ANTIPNEUMOCOCCAL RABBIT SERUM, WHEN SENSITIZING DOSE (1.0 MG. TYPE 8 PNEUMOCOCCAL POLYSACCHARIDE) AND INTERVAL (ABOUT 1 HOUR) BETWEEN SENSITIZING AND SHOCKING DOSES WERE CONSTANT

TIME	REACTIONS						CONTROLS			
15 seconds	++*	++*	++*	++	++*	++	See Text			
30 seconds	AD	AD	AD	AD	AD	AD				
60 seconds	++	AD	AD	++	AD					
90 seconds	+	AD	++	++	++	++	AD	++	AD	AD
180 seconds	+	+	0	0	0	+	+			
300 seconds	0	0	0	+	+	0				

+ = Slight anaphylactic reaction = ruffled hair, sick, expiratory dyspnea.
++ = Moderate to marked reaction = quite sick, convulsive movements, marked dyspnea.
AD = Anaphylactic death, Necropsy = pale, ballooned lungs, scattered hemorrhagic areas, bronchial exudate.
0 = No reaction.
* = Died in 24-36 hours.

As controls, four pigs were given 0.5 ml. of type 2 antiserum in fifteen seconds. Type 8 polysaccharide was used as the sensitizing dose. There were no reactions.

DISCUSSION

The conclusion is obvious, that small shocking doses of antiserum are highly effective in producing reversed passive anaphylaxis when given rapidly, whereas the same dose is essentially ineffective when given slowly. The quantitative results probably would be different if the doses of sensitizing polysaccharide or shocking antiserum were varied or if different batches of antiserum were used.[3, 4]

This experiment would seem to justify the recommendation that therapeutic antipneumococcal serum be given very slowly, in order to lessen the likelihood of reversed passive anaphylactic reactions, when tests for sensitivity are negative. Cecil and Sutliff[5] noted that "allergic reactions have nearly always occurred after the first intravenous injection of 5 c.c. or less of serum." When tests for sensitivity were negative their arbitrary "rule was to devote five minutes to the injection of 5 c.c. of serum." A safe speed in man cannot be stated from present data. In the guinea pig it is dangerous to inject 0.5 ml. (1,700 units) of serum in less than one hundred and eighty seconds.

SUMMARY

1. Evidence is presented that reversed passive anaphylactic reactions in guinea pigs vary with the speed of injection of shocking doses of specific antiserum, when other factors are constant.

2. Severe reactions occurred in most animals when the injection time was ninety seconds or less. Reactions were negligible or absent when the injection time was one hundred and eighty seconds or longer.

3. It is recommended that therapeutic antisera be injected very slowly if reversed anaphylactic reactions are to be avoided.

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CLINICAL EFFECTS OF EPINEPHRINE BY INHALATION*†

A Survey

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THE employment of epinephrine by spraying it into the tracheobronchial tract is not new. In England, Camp,[1] as early as 1929, compared the efficacy of this drug by inhalation with that of ephedrine, glycerine, and chloratone and recommended it as a valuable agent for relief of the asthmatic paroxysm. He added that extensive study had already been made in Germany on the atomization of solutions by oxygen under pressure.

The introduction into this country of the spray method for administering epinephrine seems to have originated mainly with certain relatively obscure individuals in the Pacific Northwest in the early part of the last decade. They appear not to have contributed to the regular medical journals, but have formed companies to produce a racemic brand of epinephrine and a fine nebulizer for administration.

The further development of this product is familiar to physicians who have practiced in the Pacific Northwest. The kit has consisted usually of the following three items neatly packed in a case and selling at $15.00: a bottle reputed to contain racemic epinephrine of 1:50 concentration; another small bottle containing ingredients for oral use; and an effective all-glass nebulizer. The product has appeared under a variety of trade names, usually differing for each important city, and even changing for a given locality. It has been offered for sale over drug counters in violation of local regulations, and has even been peddled at the doorsteps of asthmatics whose names had been secured. Eventually these merchandizing methods became less necessary. Asthmatic sufferers who had experienced symptomatic relief from the spray informed their friends and neighbors and directed them to a drugstore which would furnish the product without prescription. The sale increased and eventually some physicians prescribed it, and even procured it for their own relief.

It was stated on supposedly reliable authority several years ago that 3,000 persons in Portland, Oregon, alone, were users of one particular brand of this product. The interstate traffic in these Northwest brands has spread to the whole West and has to a lesser extent infiltrated through the eastern section of the United States.

It is not surprising that a reactive effort has been made by certain reputable allergists to legitimize this type of medication by securing a standard brand of laevorotatory epinephrine of titer 1 per cent and making it possible for physicians to prescribe it, together with a suitable nebulizer. Thus Glaeser and Rowe,[2] and Matzger[3] described such nebulizers and reported satisfactory relief from the intratracheal instillation of 1:100 epinephrine therewith. Various recent

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textbooks on Medicine, Allergy, and even Pharmacology, in this country and England, have published glowingly on this method of antiasthmatic medication, and have for the most part cited no contraindications.

With the more and more widespread use of nebulization and particularly the increasing popularity of aerosol, a proper evaluation of the effects of intratracheal epinephrine assumes importance. Information may be obtained from a questionnaire directed to users; from data obtained by clinical study of users and nonusers, including x-ray, fluoroscopy, bronchoscopy, and, especially, routine symptoms; the relative mortality figures in a large series, with postmortem studies. We have attempted all of these. We hope later to undertake an experimental study of animals similarly treated, in amplification of research already reported by others.

In 1942 we mailed a questionnaire to a considerable number of our asthmatic patients inquiring in detail as to any spray procedure they might have followed, and, if the answer was in the affirmative what the effects were, both favorable and otherwise. We received 115 replies. Various shades of opinion were represented, but are difficult to reduce to figures. A decided majority reported prompt relief of paroxysms. Of these some stated that the freedom was of brief duration. A large number complained that the relief of dyspnea, adequate at first, became eventually less complete; the necessary number of inhalations became greater and the intervening periods shorter; finally the benefit in many instances became negligible; and it was in these that unfavorable symptome such as weakness, palpitation, nervousness, and gastric distress made their appearance. It is obvious from the replies to this questionnaire that, as tolerance to the drug became established, larger and larger dosage was employed. Correspondingly, local symptoms such as cough made their appearance. Moreover, since the medication was usually not prescribed by a physician, the list of users includes instances of hypertension and hyperthyroidism, and even tuberculosis. Thus, although the harmful effects from overuse of the spray concern mainly the topical effects of epinephrine on the bronchial tree, systemic damage has in some proved serious.

The information gained from both the questionnaire and directly from patients has disclosed that individuals use the inhalation in variable degree. A minority have taken a "few whiffs" when they felt the paroxysm coming on, but have otherwise avoided it. These are in general the ones who have tolerated the medication well. Others have resorted to it dozens of times during the day and night, and breathed it intensively. There have been many who have admitted using a pint or even several pints of this powerful 1:100 or 1:50 epinephrine in the course of a year or a few years. One of our patients reported that when he complained to the promoter of the spray outfit that he no longer obtained relief from prolonged use, he was advised to continue inhaling until he did get relief, and then take a "dozen extra whiffs."

During the past ten years the authors have encountered in their private practice 2,236 cases of asthma, both extrinsic and intrinsic, and including those designated as bronchitis with asthma. So-called cardiac asthma has been excluded from our tabulation. A careful record has been attempted of all who,

with, or more often without, the advice of a physician, have resorted to the intratracheal use of epinephrine by nebulizer. Of these our records disclose that 648 have employed this type of therapy in varying degree, and, with two exceptions, without our approval. Early in the history of this nebulization régime one of us (R. L. B.) had already become apprehensive of harm from the prolonged vasoconstrictor action of the epinephrine. Hence our avoidance of its use. Correspondingly our attitude toward it has been critical.

Of the 648 patients whom we know to have used the nebulizer, our records show that 48 have died. We have, of course, no certain way of determining how many more may have met a similar fate, since our practice is scattered over several states. The number of autopsies (10) is proportionately low, partly from the same reason. We have, however, gained all possible data from the autopsies, and have endeavored to learn a great deal more from our clinical studies, often with the aid of roentgenography, fluoroscopy, and bronchoscopy. Equally instructive have been the hospital studies of a large number of spray users who have survived serious illness with findings similar to those of patients observed at autopsy. These non-fatal cases will be considered in detail in a subsequent report.

FATAL CASES

CASE 1.—*Complaint and History.*—B. M., a 35-year-old man, single, textile worker in a woolen mill, consulted us Jan. 27, 1942, complaining of asthma following pertussis at the age of 11 years. Attacks had increased in intensity through the years, attended by cough which had recently become dry and unproductive. During less than seven weeks under our care he had always appeared cyanotic, and coughing had been more pronounced than wheezing.

Physical Examination.—This revealed a well-developed, somewhat obese male. The skin was warm and moist but cyanotic. Examination of the chest disclosed evidence of emphysema, with numerous coarse musical rales in both lung fields. Heart borders were within normal limits, sounds normal, pulse 112. Blood pressure was 122/78.

Laboratory Examinations.—Blood was normal except for eosinophilia of 5 per cent. Sputum had 12 per cent eosinophiles, and a flora of Alpha and nonhemolytic streptococci. Stool culture yielded numerous Alpha and Beta streptococci in the third plate on the decimal scale of dilution with blood agar, along with the usual *E. coli.* The patient reacted moderately to extracts of housedust, feathers, and several other epidermals.

Clinical Course.—Cough and cyanosis continued, being always out of proportion to the wheezing. Questioning disclosed that the patient had acquired an epinephrine nebulizer without prescription two and one-half years previously, and had used it intensively night and day. At first it had given relief, but had gradually lost its efficacy. Still he had continued to employ the drug in increasing amount and frequency.

On March 13, 1942, he became acutely ill and remained home from work. Examination disclosed a temperature of 103° F., extremely rapid pulse, severe cough, and increased cyanosis. He was promptly hospitalized and given oxygen inhalation and other medication for his dyspnea. During the thirty-two hours in the hospital it became increasingly evident that he was getting inadequate oxygen into his lungs. The rapidity of his decline precluded bronchoscopy, and he died. When the mother was approached for consent to an autopsy, she disclosed that the patient had broken his fine nebulizer and had subsequently inhaled the racemic epinephrine through a coarse DeVilbiss atomizer. It is our befief that this substitution greatly increased the amount of the drug introduced into the bronchial tree, and thereby hastened the fatal result.

Autopsy.—The arterially embalmed body was sectioned by Dr. J. D. McGovern and reviewed by Prof. Warren C. Hunter, of the University of Oregon Medical School. We need

recite here only the findings in the respiratory tract, since, outside of general passive hyperemia and right cardiac dilatation, there was no other pathology contributory to death.

The mucous membrane of the trachea was found deceivingly smooth and of normal color. The lumen of its lower half was, however, densely filled with a plug of greyish-white, friable, gelatinous material which extended continuously into both main bronchi, and on into their branches. On cutting through each lung similar plugs of material were found filling the lumina of the smaller bronchi, but these assumed a more definitely purulent appearance than in the larger bronchi. Other gross changes in the lungs included bilateral hyperemia and edema; areas of atelectasis; and, in the right lung, areas of pneumonic consolidation. Hydropericardium and bilateral pneumothorax were present.

Microscopic.—Because of the importance and uniqueness of this case, the changes can best be expressed in the original language of the pathologists. Sections were made from three levels of the trachea, from above downward; from bronchi of various sizes; and from lung parenchyma.

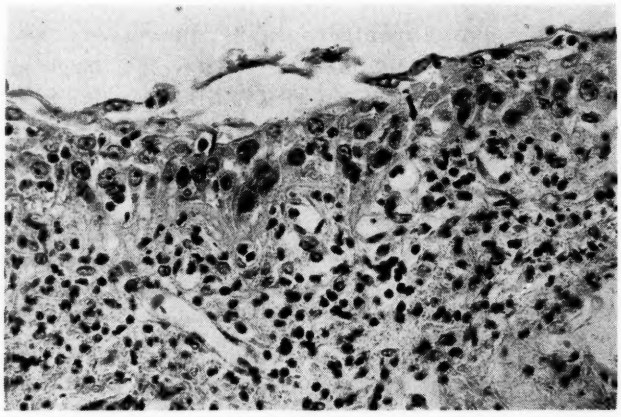

Fig. 1.—Microphotograph of wall of trachea of Case 1 displaying complete metaplasia of epithelium to stratified squamous type with desquamation of cells.

The epithelium over the posterior aspect of the upper portion of the trachea still retains its normal pseudostratified columnar appearance, but the submucosa contains a light infiltration of scattered lymphocytes and monocytes, with an occasional plasma cell and polymorphonuclear leucocyte. The epithelium of the lateral and anterior aspects, however, reveals an entirely different picture (Fig. 1), in that the epithelial surface is flattened, and tends for the most part to be composed of three or four layers of pavementlike cells entirely devoid of cilia. In not a few instances the superficial layer shows true and typical keratinization, with fading out of the nuclei, increased eosinophilic staining and opacity of the cytoplasm. Throughout the submucosa in these portions the cellular infiltration is more dense, and here tends to be quite heavy in the zone immediately underlying the altered epithelium. In occasional instances small areas of desquamated keratin material are encountered. At occasional points along the surface epithelium there is a break in the continuity, and the surface exposes newly formed vascular channels, some of which contain red blood cells. It is in the vicinities of these areas of ulceration that one notes the more dense accumulation of cells which include lymphocytes, monocytes, fibroblasts, and occasional plasma cells and polymorphonuclear leucocytes. Also in these areas and throughout the greater portion of the submucosa the presence of newly formed collagenic connective tissue is readily apparent. The inflammatory exudate at no point extends into or around the mucous glands. In lower levels of the trachea the inflammatory exudate becomes more abundant and polymorphonuclear leucocytes more numerous, and there is more evidence of hyperemia and edema. The areas of ulceration are more extensive, with more epithelial desquamation and denuding of the underlying vascular tissue.

In the main bronchi (Fig. 2) ulcerative changes become more extensive, and there is early desquamation of large areas of the stratified squamous and partially keratinized epithelium. Masses of fibrin in places overlie the denuded and granulating surface. Dilatation of blood vessels becomes more striking, and cellular exudate is invading more deeply into the mucous glands. The changes described continue downward through the bronchi of the first, second, and third order. Epithelial metaplasia is almost everywhere present, with 2 to 3

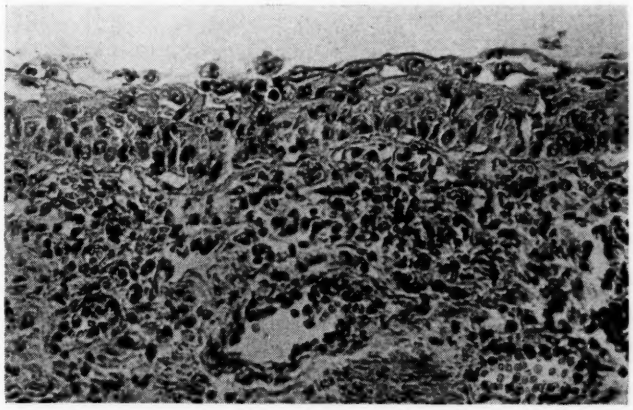

Fig. 2.—Microphotograph of wall of main bronchus of Case 1 displaying metaplasia of epithelium with cellular infiltration of mucosa and submucosa.

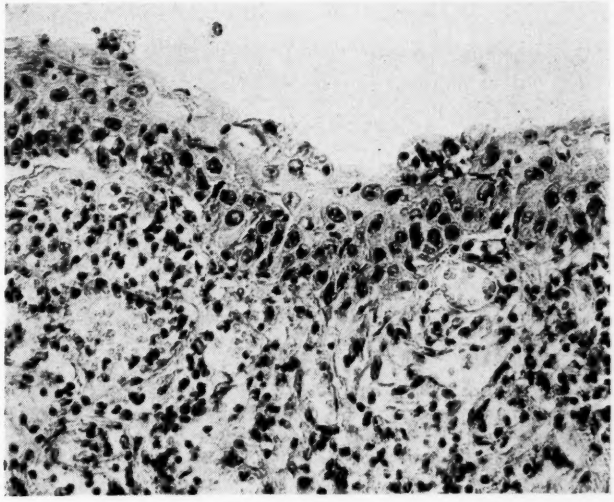

Fig. 3.—Microphotograph of mucosa of bronchus of first order, Case 1, displaying metaplasia of epithelium.

irregular layers of flattened cells (Fig. 3). Submucosal abscesses are in places discharging their contents into the lumen to form fibrinopurulent plugs containing desquamated epithelial cells and detritus (Figs. 4 and 5). In the parenchyma from peripheral portions of the lung the smaller bronchi and bronchioles display almost complete destruction by inflammatory exudate, and occlusion of their lumina by plugs of mucoidlike material and leucocytes. The parenchyma itself, especially of the right lung, contains dense areas of pneumonic consolidation.

Comment.—It is apparent that the gross; and especially the microscopic, findings in this case, in correlation with the clinical history, present a unique picture. Had this patient intentionally sacrificed himself for experimental study, he could not possbly have offered a clearer demonstration of the extreme pattern of tissue injury from excessive bathing of the

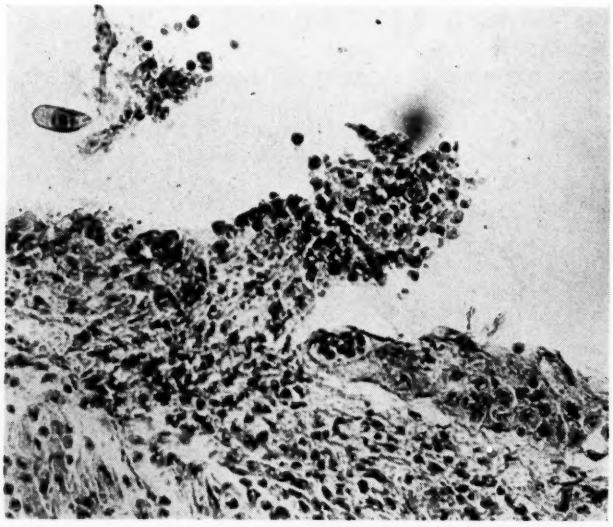

Fig. 4.—Microphotograph of bronchial mucosa of Case 1, displaying metaplasia of epithelium and rupture of subepithelial abscess into lumen.

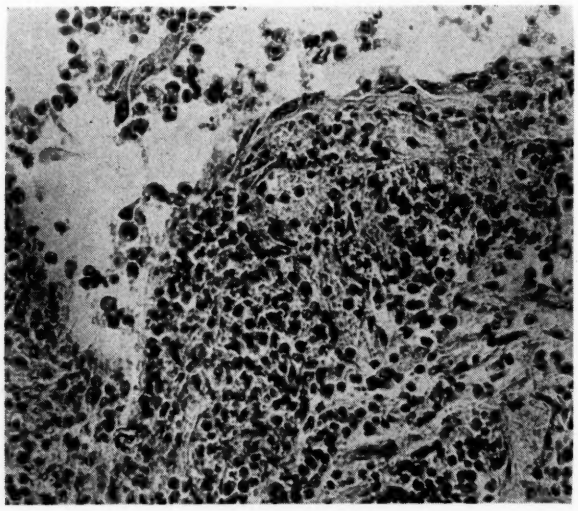

Fig. 5.—Microphotograph of wall of bronchus of third order, Case 1. The stratified squamous epithelium is desquamating into lumen, along with ruptured subepithelial abscess.

delicate respiratory mucosa in concentrated epinephrine solution. Substitution of a coarse atomizer, with its heavy spray, for the fine nebulizer originally supplied with the outfit, has resulted in a drenching of the mucosa, even to the finer branches of the bronchi.

The results, in order of their occurrence, may be conceived to be about as follows: ischemia of the tissue; local damage to epithelium with loss of cilia; metaplasia of epithelium

from columnar to pseudostratified with early keratinization and desquamation; interference with the ducts of the mucinous glands; cellular infiltration of subepithelial layers, mainly by mononuclear cells; formation of microscopic subepithelial abscesses and rupture of these into the lumen of the bronchial tree; and formation of plugs consisting of fibrinopurulent exudate, cellular detritus, and mucin. End results vary from areas of atelectasis to a complete acute obstruction of the bronchial tree.

This case has been presented as the extreme. One other patient made the same blunder of using a coarse atomizer, but fortunately became nauseated and discontinued the spray. The further cases that we shall submit will concern the use of epinephrine by fine nebulizers. The effects will be found correspondingly milder and their morbid course more protracted.

CASE 2.—M. K. M., a 43-year-old woman, first came to the office on Oct. 7, 1946, because of perennial nasal obstruction of 20 years' duration, and asthmatic symptoms for the preceding 4 years. Nasal polypi had been removed several times, and she had been following an allergic program of elimination of various inhalants, and hyposensitization with house-dust and orris root. In spite of this her asthma became progressively worse, with almost continuous mild dyspnea and coughing between the acute paroxysms. Among the various medicaments for symptomatic relief was included epinephrine by inhalation, which in more recent months she had used several times an hour. At this time neither she nor her husband expressed alarm over any sudden change in character or severity of her symptoms, yet she appeared to us as a patient who was gravely ill. The oral temperature was 102° F., respirations were very rapid and shallow, lips and nail beds were cyanotic, and chest findings suggested diffuse parenchymal involvement. She was immediately hospitalized and provided with supportive and symptomatic treatment, including oxygen and penicillin. At first she improved slightly, and cyanosis was controlled, but within 24 hours she became extremely toxic with little or no febrile course, and in spite of heroic measures went rapidly downhill, expiring on the fourth hospital day. During the short period of observation a necrotic adherent membrane was noted on the posterior pharynx. Although the culture was negative for *C. diphtheriae*, 20,000 units of diphtheria antitoxoid were administered. The chest x-ray confirmed the auscultatory findings of patchy consolidations throughout both lung fields.

Autopsy.—Grossly the lungs were diffusely studded with varying sized nodules, which on sectioning were evidently areas of consolidation having the configurative pattern of the smaller bronchioles. On compression these smaller bronchioles exuded greenish-yellow purulent material.

Microscopic findings were more enlightening. In the trachea many areas showed marked edema and fragmentation of the mucosa and widespread inflammatory infiltration in the subepithelial area. This exudate was predominantly mononuclear in character. There was an extensive mild fibrosis underlying the epithelium. In some areas in the neighborhood of the vocal cords, the epithelium was eroded and replaced by a thick, granuloma-type membrane. Multiple sections through lung tissue showed a generalized consolidation with obliteration of alveolar spaces. Occasional bronchi were identified, in which the epithelium was replaced by necrotized pyogenic membrane which exhibited no evidence of organization. For the most part, the consolidation was represented by massive fibrinous precipitate in which cellular activity was minimal and in which early organization could be identified. Occasional zones where the septal walls were broken down had confluent masses of pus cells, epithelial debris, and fibrino-cellular exudate suggesting miliary abscesses. In the occasional areas where the alveolar spaces were empty, interstitial thickening was evident.

Comment.—In this case, where nebulized epinephrine was used frequently, interesting acute and chronic tracheal, bronchial, and alveolar processes are well demonstrated. The subtle course leading to an overwhelming pyogenic process is here well illustrated.

CASE 3.—This patient, L. S. P., a male physician, aged 28 years, came to our office Jan. 6, 1938. He had suffered recurrent nonseasonal asthmatic seizures from early childhood, usually following acute upper respiratory infections. The attacks had formerly responded readily to ordinary relief medication. He received inoculations for several months with autog-

enous vaccine prepared from his sputum and stool cultures; also with extracts of household dusts to which he had reacted. In June of that year he discontinued treatment in our office, and was free from asthma.

In the winter of 1938-1939 the patient's asthma recurred, and attacks continued intermittently, associated with chills and fever. He eventually limited his treatment to self-medication, including a commercial oral spray of racemic epinephrine similar to that mentioned in Case 1. At first this inhalation gave relief, but its effectiveness gradually diminished and at the same time the frequency and intensity of administration increased.

In the autumn of 1941 he accepted a hospital residency. The work was burdensome and the patient wished to conceal his dyspnea. Thus he resorted, along with ordinary medication, to more intensive use of the spray. In the fatal episode, while on a trip, he ate a good dinner, experienced discomfort, and retired to his room to use the nebulizer. At his request his wife then gave him adrenalin 1:1,000, 0.5 c.c., by hypodermic injection; fifteen minutes later another 0.5 c.c.; one-half hour later 1.0 c.c., and soon another 1.0 c.c.; then, after being carried into the hospital a physician gave him 0.75 c.c. adrenalin and grain ¼ morphine. Cyanosis and extreme tachycardia followed as unconsciousness developed. After having exuded frothy material from his mouth for several hours, the patient died (June 1, 1942).

Autopsy.—Anatomical diagnosis was: asthma, emphysema, acute purulent bronchitis, and early bronchopneumonia, pulmonary edema, and cor pulmonale.

Microscopic.—The histologic picture is obscured somewhat by embalming, but indicates lack of epithelial metaplasia in the trachea and bronchi. Sections from the main bronchi and those of the second and third order display plugging of their lumina by exudate consisting of mucus with inclusion of neutrophilic granulocytes and clumps of bacteria. In places granulocytes can be seen escaping through the epithelial layer into the lumen. The muscular layer is hyperplastic and there is edema and inflammation of the submucosa, the invading cells here being mainly eosinophile granulocytes, with few neutrophiles and lymphocytes.

Comment.—The administration of 3.75 c.c. of epinephrine 1:1,000 subcutaneously within a short period, followed by subcutaneous injection of grain ¼ morphine may have played an important role in this patient's sudden and dramatic death. Its only importance to the subject of this report is in that it provided autopsy material from a patient who had suffered from asthma for 4 years and had inhaled a concentrated racemic mixture of epinephrine excessively for about 3 years. The pertinent mucosal and submucosal changes are less marked and less extensive than those seen in Case 1. Exhaustive search failed to reveal any epithelial metaplasia. It is reasonable to compare the relative degree of pathological changes of the two in the light of the extent to which the tracheobronchial mucosa of each case was exposed to concentrated solution of epinephrine.

It is of interest that two of our fatal series displayed an ulcer of the duodenum at autopsy. The reader's attention is also directed to the animal studies of Galgiani et al,[4] who observed diarrhea, anorexia, and loss of weight in two cats which were fed 1.0 c.c. of 1 per cent epinephrine solution 6 times a week for different periods of time. Both at autopsy showed inflammation of the intestinal serosa together with many small ulcerated and hemorrhagic areas of the mucosa. Another cat which received inhalations of 1 per cent epinephrine twice daily died on the sixth day and showed similar changes. It seems possible, therefore, by analogy that the swallowing of epinephrine in the course of excessive and prolonged inhalation might produce somewhat the same pathology in the human being.

It is impossible in this brief report to present a complete protocol of each of our 48 known fatal cases. Many have died in distant localities, and the immediate cause of death has not always been learned. It is of value, however, to summarize such information as may be gained from our own and hospital records and outside sources, and the deductions thus gained will allow us to arrive at a helpful conclusion.

The respective ages of our subjects at the time of death are worthy of consideration. These range from 27 to 75 years, and the average is 54 years. Only three were 70 years or older. Despite the high average in age at death, in few instances can senile decline be assigned the major role. Fifteen were under 50 years, and on the lower end of the age scale we have seen definite evidence of harm, though not fatal, in infants and young children from

the inhalations. Thus Baby S. J., aged 19 months, was seen by one of us at the bedside with extreme prostration, cyanosis, and unproductive cough following excessive inhalation of epinephrine at the hands of his grandmother. Discontinuance of the spray, together with proper medication, relieved all of the symptoms, including asthma.

Obvious contraindications to the use of epinephrine appear in several of the fatalities. Mrs. W. G. R., a proved asthmatic with hypertensive cardiorenal disease, blood pressure 212/128, and an excessive addict of the spray, died of reported "stroke" at the age of 48 years. Mrs. W. T. P., an extrinsic type asthmatic, with a blood pressure of 208/128, died at the age of 67 years following prolonged use of the spray, and W. D., male, with B. P. 212/108, at 58. Two other mild hypertensives among our list of habitués died at the ages of 61 and 63 years. Otherwise most of our fatal cases exhibited blood pressures ranging from 90/50 upward through the normal range. In a few instances the systolic pressure increased by 20 to 37 points following continued inhalations, but the diastolic increase was smaller.

Five patients, R. G., aged 65 years; J. A., aged 56 years, J. H., about 50 years; D. C. H., 57 years; and Brother D., 65 years; all males, ended in sudden death suggestive of coronary disease, but unconfirmed by autopsy. Mrs. J. S. V., 62 years, died in anasarca, unexplained.

Two are recorded as postoperative fatalities. D. B., a male, 56 years, developed cough, dyspnoea and bronchial obstruction following appendectomy and died the same day. The second, Mrs. J. W. C., died while emerging from anesthesia of an operation for carcinoma of the cecum.

DISCUSSION

The foregoing citation of 48 instances of death following inhalation of epinphrine is, we hope, self-explanatory. We have stated the known facts in each case, and have refrained from ascribing each death implicitly to a certain form of medication. We cannot, however, escape the conviction that in certain cases epinephrine, used topically on the respiratory mucosa in a concentration of 1:100, and with sufficient intensity and frequency, has resulted in serious local damage; and that the resultant systemic effect has, through destruction of the respiratory epithelium and subsequent formation of occluding plugs, terminated in anoxia and death. We shall, therefore, summarize the evidence supporting this contention.

Clinical Pattern of Harm.—Most striking perhaps is the preponderance of statistical evidence. We have compiled 48 known deaths among 648 users of spray in the past ten years in a total of 2,236 asthmatics treated in that period. During the same time there have been 22 deaths among 1,588 nonusers of the spray.

Another strong evidence of harm from excessive and prolonged inhalation of the drug has come from clinical study of the subjects. Confirmed users have usually exhibited a cough which was persistent, dry, and out of proportion to the intensity of the asthmatic symptoms. Immediately after an intensive spraying the cough has tended to become more productive, and often resulted temporarily in the raising of mucus, but the cough has persisted. Cyanosis is predominant and weakness, sleeplessness, irritability, nervousness, tachycardia, palpitation, and gastric distress with nausea and vomiting have been common complaints. The patient has usually been aware of sore throat, and inspection has disclosed edema and hyperemia of the pharynx. Bronchoscopy has revealed congestion of the trachea and primary bronchi, and, in many cases, firm plugs

which were difficult to dislodge. The associated infectious processes have included trachebronchitis, bronchiolitis, pneumonitis, submucosal abscesses, and even interstitial pneumonitis. In many instances these have appeared clinically without the patient's awareness of the development of a new process other than increased resistance of asthmatic symptoms to symptomatic treatment. Chest roentgenograms and fluoroscopy have commonly revealed an ill-defined patchy density, often referred to as pneumonitis, but probably representing multiple areas of atelectasis resulting from bronchial plugs. Low-grade pneumonia, whether or not detected by roentgenogram, has usually presented itself at autopsy.

One of the most universal clinically diagnostic signs of overuse of this drug is the patient's acquired tolerance, not only to the drug so administered, but also to the hypodermic route. Eventually he becomes refractory to other forms of medication as well. Even oxygen may be relatively ineffectual in that it fails to reach those segments of lung parenchyma whose bronchi are plugged. Relief becomes difficult and convalescence at best is slow.

Pathologic Pattern of Harmful Effects.—Case 1 has afforded us a grim pattern of the most extreme harm which can result from continually bathing the mucosa of the bronchial tree with concentrated epinephrine. The coarseness of the improvised atomizer had allowed the vasoconstrictor drug to permeate the trachea and its branches, even to bronchi of the 3rd and 4th order. The result has been the series of extreme changes described in the autopsy report. Further autopsies have demonstrated some of these same changes in lesser degrees. We may, therefore, give as the anatomical pattern of harm from epinephrine by spray the succession of changes listed in the comment under Case 1.

It should be mentioned, however, that this entire pattern does not appear in each case. For instance, epithelial metaplasia occurs particularly in cases where the inhalation has been most excessive; loss of cilia is probably no more frequent than reported by Hilding[5] in his careful clinical and pathologic study of asthma with asphyxia, and the cellular infiltration of subepithelial layers probably does not always progress to the extent of abscess formation, with rupture into the lumen. It may be stated, however, that ischemia of the mucosa, with resulting submucous infiltration, epithelial injury, and desquamation, and the development of plugs in the lumina of the trachea and bronchi, with eventual atelectasis, are the commonest findings in our series. Finally the histology in our more typical cases has been characterized throughout by a relative absence of eosinophilia in the pulmonary tissue involved, and a predominance of lymphoid cells, plasma cells, and neutrophiles in the mucosa and submucosa. And pneumonia has usually made its appearance, starting in the peripheral portion of the bronchial tree. Either from plugs occluding the major branches of the bronchial tree, or from widespread filling of the respiratory bronchioles with purulent exudate, the end result is essentially the same. Anoxia completes the clinical picture.

The effects related in this presentation are generally in accord with the results of animal experimentation. As early as 1922 Sollmann and Brody[6] re-

ported that topical application of epinephrine to the ears of rabbits greatly reduced the local resistance to pyogenic infection because of the ischemia produced. They also referred to Auer and Meltzer[7] in this regard.

In 1939 Fox[8] reported denuding of the nasal mucosa, leucocytic infiltration and intra-mucosal abscesses by spraying rabbits intranasally with 1:1,000 epinephrine. In the same year Galgiani et al,[4] working with cats and rabbits, sprayed the animals orally with 1 per cent epinephrine twice daily 6 days a week for a maximum of one hundred and twenty-one days for the rabbits and seventy-one rays for the cats. In some of the animals autopsy and microscopic examination revealed loss of tracheal cilia and desquamation of tracheal and bronchial epithelium; also a submucosal infiltration of the trachea with leucocytes. They obtained similar effects in the trachea of a human subject who was in the terminal states of tuberculosis.

In 1941, Hunnicutt[9] used a comparable technique with mice, spraying various vasoconstrictor drugs into the nasal tracts of mice, and obtained various grades of inflammatory reaction.

We are not attempting a survey of the pathology of asthma. We are aware that the changes which we have described can be found in the classical surveys of the pathology of asthma such as those of Huber and Koessler,[10] in 1922, Thieme and Sheldon[11] in 1938, Steinberg and Figley,[12] in 1928, and Hilding,[5] in 1943, and for this reason we have not attempted to review the literature on that subject. The fact that changes which we have described were found at autopsy long before epinephrine by inhalation was used can scarcely discredit the role of this drug in the development of such pathologic changes. The tissue of the respiratory tract has the capacity to react in a definite pattern when injured, whether by bacterial or chemical action. The experimental studies on animals and man[4] clearly proved this. It is reasonable, therefore, to believe that the diseased respiratory tissue of the asthmatic, with its high bacterial flora, when repeatedly exposed to the effects of epinephrine will be easy victim to accelerated pathological processes.

SUMMARY

1. A clinical study and information gained from a questionnaire indicates that epinephrine used in a concentrated form by oral inhalation usually offers symptomatic relief of asthma. This relief in most cases diminishes with continued use of the method, and more frequent and intensive administration becomes necessary. Serious harm results from excessive use.

2. An attempt has been made to describe the clinical and pathological changes which we have observed in our practice from excessive spraying. This includes a consideration of 48 private patients who have died following prolonged employment of this method of self-medication.

3. Our survey of 2,236 asthmatics in our practice in the past ten years gives the following mortality figures. Among 648 users of the oral epinephrine spray, 48, or 7.4 per cent, have terminated fatally. Among 1,588 supposed nonusers of this form of medication, 22, or 1.4 per cent, have died. Since the mor-

tality in users was over five times as great as in nonusers, we believe that injudicious use of this form of medicinal relief has contributed materially to death from asthma in our series.

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A CLINICAL INVESTIGATION OF BETA-MORPHOLINO-ETHYL BENZHYDRYL ETHER HYDROCHLORIDE (LINADRYL) AS AN ANTIHISTAMINE AGENT*†

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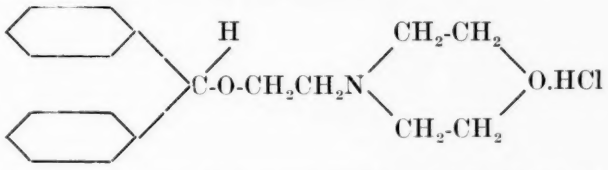
BETA-MORPHOLINO-ETHYL benzhydryl ether hydrochloride, one of the alkamine benzhydryl ethers, hereinafter called "linadryl," is closely related chemically to beta-dimethyl-amino-ethyl benzhydryl ether hydrochloride (benadryl), as the following structural formula indicates:



Linadryl has been shown to possess antihistaminic and antispasmodic properties[1-3] similar to benadryl. However on the basis of study with small laboratory animals it is probably less than half as effective as benadryl, and has shown a correspondingly lower toxicity in both acute and chronic intoxications.[1-3] Because benadryl has had a tendency to produce unpleasant symptoms in approximately 25 per cent of those to whom it has been administered,[4] the present study of linadryl has been undertaken.

MATERIALS AND METHODS

Eighty-two men from 19 to 80 years old, with an average age of 41.2 years, and 168 women from 14 to 81 years old, with an average age of 37.3 years, have served as the subjects of this study. These individuals were divided into two major groups: (1) those who had no known or recognizable disturbance of the autonomic nervous system, and (2) those in whom allergic disease existed, or in whom a disturbance of the balance of the autonomic nervous system was recognizable. The former group consisted of 34 men and 73 women. Of these, seven (five men and two women) were subjected to a complete cycle of examinations designed to appraise the influence of linadryl upon major systems and organs of the body in a manner detailed elsewhere in connection with our studies of benadryl.[4, 5] In the remaining 100 subjects of this group, a controlled study of "side reactions" to linadryl was carried out. All patients received the drug for not less than two weeks, nor for longer than twenty weeks.

The major diagnosis encountered in each of the 143 patients included in the second group is recorded in Table I.

*From the New York Medical College, Metropolitan Hospital Research Unit, and the Department of Medicine, New York Medical College, New York, N. Y.

†Generous supplies of this material were made available through the courtesy of Dr. E. A. Sharp, Director of Clinical Investigation, Parke, Davis & Co., Detroit, Mich.

TABLE I. RESULTS OF TREATMENT WITH LINADRYL AND A COMPARISON OF ITS EFFECTIVENESS WITH THAT OF BENADRYL

DIAGNOSIS	NO. PTS.	DOSE OF DRUG (MG.) DAILY RANGE	DAILY AVERAGE	NO. DAYS RANGE	NO. DAYS AVERAGE	RESULT COMPLETE RELIEF	RESULT IMPROVEMENT	NO RELIEF NUMBER	NO RELIEF PER CENT	COMPARISON WITH BENADRYL NO. PTS.	EFFECTIVE IN 2 X DOSE	EFFECTIVE IN >2 X DOSE	NOT EFFECTIVE	PER CENT SIDE-EFFECT	REMARKS
Seasonal hay fever	50	150-400	275	12-70	49	12	8	30	60	8			8	8	
Vasomotor rhinitis	9	50-300	250	14-150		2	4	3	33	5	4	1		33	P.B.Z. ineffective at 200 mg. level in 1 responding to 300 mg. Lin. Side effect. sl.
Urticaria (a) Acute	8	100-500	400	Unit-3	1.5	6	2	0	0	0				0	
(b) Chronic	14	300-600	500	6-70	13	3	8	3	21	5		4	1	21	
Atopic eczema	9	300-750	500	9-120	67	0	6	3	33	1		1		11	
Bronchial asthma	49	200-750	400	7-120	80	5	17	27	55	4	2	2		12	1 preferred this drug to Benadryl 1 > Lin. had N.R. fr. ½ dose P.B.Z.
Neurodermatitis	3	300-400	367	14-70	29	0	1	2	67	1			1	0	
Spastic colon	1	400	400	4	4	0	0	1	100	1			1	0	
Total	143					28	46	69	48.2	25	6	8	11		

RESULTS

Analysis of Data Obtained on Subjects Without Disturbance of the Autonomic Nervous System.—Weight, basal metabolism, respiratory function, pulse rate, cardiac activity, blood pressure, circulation time, renal function, gastrointestinal motility, morphologic elements of the blood, hematocrit, hemoglobin, and several chemical determinations upon the blood, including non-protein nitrogen, urea nitrogen, creatinine, glucose, glucose tolerance, protein, albumin-globulin ratio, total and free cholesterol, alkaline phosphatase, icteric index, Van den Bergh reaction, and cephalin flocculation, yielded information well within the normal range. Positive actions of the drug in our "normal" subjects included:

1. *Alterations in the Central Nervous System.*—Drowsiness and other disturbances of the sensorium will be discussed under "side reaction." However, 25 of the 107 subjects that belong to Group I reported an improvement in sleep and a more placid outlook on life while taking the drug. Careful appraisal revealed that these 25 subjects were among the more sensitive and "high strung" of the group, although showing no frank imbalance of autonomic nervous system activity.

2. *Alteration in Gastric Acidity.*—Thirty-seven fractional gastric analyses were performed on 7 patients before and after receiving Linadryl at various dosage levels for periods varying from one to several weeks. The percentage changes noted in Table II were derived by treating each subject as his own control. The abscissa of the curve is plotted as time from 0 to 90 minutes, and the ordinate as units of gastric acid. The "area of any given curve" represents that space contained between the 0- and 90-minute ordinates, and the zero line and plotted curve of units of acid, respectively. The area of the curves obtained prior to the administration of linadryl were arbitrarily fixed at 100 per cent in each instance, and the "test" curves calculated as a percentage thereof (Table II). By this method, values between 90 and 135 per cent were not significant statistically.

TABLE II. VARIATIONS IN GASTRIC ACIDITY FOLLOWING THE USE OF LINADRYL

	NO. PTS.	DOSE OF LINADRYL		AVERAGE AREA OF TEST CURVES (PER CENT OF CONTROLS)*	
		DAILY (MG.)	TOTAL (DAYS)	FREE	TOTAL
Decreased acidity	2	400	14	30.1	49.8
	2	800	21	37.8	41.7
Unchanged	3	400	28	92.7	107.5
	3	600	23	100.4	90.7
	2	800	25	124.7	118.4
	1	1200	14	89.0	93.9
Increased acidity	1	400	14	148.0	280.7
	2	600	21	248.7	210.4
	2	900	14	230.4	250.1
	1	1200	21	190.5	215.6

*Values between 90 and 135 per cent are not statistically significant in these calculations.

3. *Decrease in capillary permeability.*—In three subjects, a "tightening" of the capillaries occurred while they were taking 600 mg. or more of linadryl. but at and above this dosage level no constant relationship between dosage and the degree of decrease in permeability could be detected.

4. *Variations in the Reaction of the Skin to Histamine.*—No constant changes in the wheal and flare reaction of the skin to histamine were observed until 600 or more mg. of drug were administered daily. In three subjects at

this level of dosage, an appreciable decrease in the size of both wheal and flare was observed. In all patients who received 800 mg. or more daily, a decrease in this response was present. In one patient the histamine reaction failed to appear at all; in five others the change was less dramatic. In one patient, who had showed a slight decrease in these responses with daily doses of the drug ranging from 800 to 1,000 mg., an actual increase in both the wheal and flare occurred after 1,200 mg. had been used for fourteen days.

THERAPEUTIC STUDY OF LINADRYL

One hundred forty-three patients with allergic diseases have been treated to date with linadryl (Table I). The drug was most satisfactory in acute urticaria, having given complete relief in every instance. However, since this is a self-limiting condition as a rule, it is difficult to appraise fully its action in this connection. That itching was markedly relieved seems clear. The drug was decreasingly successful, somewhat in the order named, in chronic urticaria, vasomotor rhinitis, atopic eczema, bronchial asthma, hay fever, and neurodermatitis. One patient with a spastic colon failed to obtain any relief from the use of 400 mg. daily for four days.

In all, twenty-eight, or 12.5 per cent of the patients treated obtained complete recession of their symptoms while under treatment with 300 to 750 mg. of linadryl daily. An additional 48.0 per cent were partially relieved of their complaints. The remaining 39.5 per cent received no benefit whatsoever from the administration of linadryl over considerable periods of time (up to one hundred twenty days).

It was possible to compare the effects of linadryl and benadryl in twenty-five patients (Table I). In all twenty-five, benadryl had been completely effective in the relief of symptoms at some dosage level. In eleven of these, linadryl was not effective at any given daily dose up to 750 mg. In eight, it required from three to four times the dose of linadryl to produce effects closely approximating those following the administration of benadryl. In six patients, twice the effective dose of benadryl or less was sufficient to bring about a relief of symptoms.

UNTOWARD REACTIONS

Untoward reactions to linadryl were looked for in the 250 patients who received from 50 to 1,200 mg. of the drug daily (Table III). No side-effects

TABLE III. UNTOWARD REACTION IN 250 HUMAN SUBJECTS RECEIVING LINADRYL

TYPE OF REACTION	REACTIONS* NUMBER	REACTIONS* PER CENT*	DOSAGE LEVEL (MG.) AT WHICH UNTOWARD SYMPTOMS WERE FIRST OBSERVED 300	400	450	500	600	750	900	1050	1200
Total patients at dose level*	277	100	137	17	35	12	21	24	12	12	7
Drowsiness	36	12.9	9	1	5	0	4	5	3	5	4
Dizziness and blurred vision	7	25.5	1	0	0	0	1	1	0	3	1
Headache	4	1.4	1	0	0	0	0	1	1	1	0
Confused sensorium	4	1.4	0	0	0	0	0	0	0	4	0
Fatigue and weakness	3	1.1	1	1	0	1	0	0	0	0	0
Loose stools	2	0.7	0	0	0	0	0	0	0	0	2
Heartburn	2	0.7	0	0	0	0	1	0	0	0	1
Dry mouth	1	0.4	1	0	0	0	0	0	0	0	0
Abdominal cramps	1	0.4	0	0	0	0	0	0	0	0	1
Palpitation	1	0.4	0	0	0	0	0	1	0	0	0
"Jumpiness"	1	0.4	0	0	0	0	0	1	0	0	0
Total	62		13	2	5	1	6	9	4	13	9

*It will be noticed that addition of the number of patients at the various dose levels totals 277. This apparent discrepancy is due to the fact that each subject is listed under every dose he received. The percentages are calculated from this total and therefore represent a weighted figure slightly higher than the actual percentage of subjects in whom an unpleasant response occurred.

were noted until 250 mg. or more of the drug was given daily. When reactions did occur, they were more likely to appear after the first dose of the drug rather than later. A tolerance similar to that observed for benadryl usually developed as the drug was continued, although at the higher levels of dosage (900 mg. and more) this was less likely to be true. Moreover, even in the largest doses, the side-effects were incapacitating in only two patients, while in the remainder they were much more mild than those seen with benadryl.

Forty-three of the 250 patients, or 17.2 per cent, developed a total of sixty-two instances of untoward reactions (Table III). As was the case with benadryl, drowsiness was by far the most common. It will be noticed that the percentage of reactions increased more or less proportionately to the amount of drug administered, thus suggesting the true toxic nature of the side-effects.

SUMMARY

1. The effects of beta-morpholino-ethyl benzhydryl ether hydrochloride (linadryl) have been observed in 107 persons without recognizable disturbances of the autonomic nervous system, and in 143 individuals with allergic diseases.

2. When administered orally in doses ranging from 300 to 1,200 mg. daily, a variable effect, irregularly related to dosage, was exerted upon the secretion of gastric acid. The histamine responses of the skin decreased after one week of treatment with 600 mg. daily in about one-third of the patients, while a routinely depressant effect was observed by a similar period of administration in daily doses of 800 mg.

3. A certain sense of well-being with more restful sleep was experienced in a group of 25 "high strung" individuals.

4. Twelve and one-half per cent of the patients with allergic disease obtained complete relief of symptoms while using linadryl in doses up to 750 mg. daily, while an additional 48.0 per cent were partially relieved.

5. In 17.2 per cent of the patients, some discomforting reactions occurred, of which drowsiness was by far the most common.

6. It is concluded that linadryl has an action similar in nature to that of benadryl, but is probably less than one-half as effective weight for weight. On the basis of percentage, it would probably cause an equally high number of unpleasant symptoms were its administration pushed to a point of comparable effectiveness in every case.

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Book Reviews

CLINICAL ALLERGY, by Alexander Sterling, M.D., New York, 1947, International Universities Press, pp. 198.

With so many books on allergy being published, one wonders what prompted the author to add still one more. The answer appears in the preface wherein the aim of this monograph is to acquaint the general practitioner and interested specialists with clinical allergy and guide them in the proper diagnoses and treatment. Discussions of immunology, anaphylaxis and allergy, and details of theory are purposely avoided.

The author proceeds to outline the diagnoses and treatment of a number of clinical disorders along lines which in his long experience he has found most satisfactory. These are exemplified by many case reports. There is a final chapter on laboratory procedures which deals essentially with the preparation of extracts. Although the work of others is referred to occasionally, this book is essentially a record of the author's personal thought and experience. Most of the material is conventional but it may have been presented fifteen years ago. Much of the bibliography dates back two decades.

Those who use this book as a text will not go astray, but it is like reading an early edition of a book when a modern one is available.

American Academy of Allergy

REPORTS OF FOURTH ANNUAL MEETING OF THE AMERICAN ACADEMY OF ALLERGY

HISTORIAN'S REPORT

The Fourth Annual Meeting of the American Academy of Allergy took place in St. Louis, Dec. 15-17 inclusive, with headquarters in the Hotel Jefferson; 110 fellows and 286 members attended.

At the opening business session, minor changes in the constitution and by-laws were adopted in the interest of clarity of statement and democracy of operation.

The office of Historian was added and the duties defined.

The following officers were elected: Karl D. Figley, president; Walter L. Winkenwerder, vice-president; Theodore L. Squier, secretary; Horace S. Baldwin, Treasurer; and Alan G. Cazort, historian.

A healthy growth in the relatively new field of allergy was manifested by impressive lists of new members and fellows. The following special elections were made: Dr. Alfred Gilman, Professor of Pharmacology at Columbia Universtiy, to honorary fellowship; Dr. Willem Kremer, of Amsterdam, Holland, directly to fellowship; and Miss Margaret Strauss, to associate membership.

Thirty-eight members were admitted to fellowship. One hundred two new members were added.

The growing desire of allergists to attain a status on a par with other specialties in medicine was expressed in a resolution introduced by Dr. George Piness, the significant part of which is given below:

> It is therefore now resolved by the fellows of the American Academy of Allergy to direct the President to appoint a committee of three to take the necessary procedures required by the Advisory Board of Medical Specialties of the Council of Education of the American Medical Association for approval and establishment of an American Board of Allergy.

The resolution was warmly debated. A vote by closed ballot resulted in a count of sixty-five for the resolution and forty-five opposed.

The following Memorial Lectures added greatly to the scientific program:

To Dr. Aaron Brown was dedicated the lecture, "The Pharmacology of Drugs in Allergic Conditions," by Dr. Alfred Gilman, Professor of Physiology at Columbia University.

In memory of Dr. J. Alexander Clarke, Jr., Dr. J. Bronfenbrenner, Professor of Bacteriology and Immunology, Washington University, discussed "Hypersensitiveness and Immunity in the Light of the Unitarian Hypothesis."

Dr. Warren T. Vaughan was commemorated by an illustrated lecture, "Hypersensitivity in the Pathogenesis of Diseases Characterized by Collagens and Vascular Damage," by Dr. Arnold R. Rich, Pathologist-in-Chief, Johns Hopkins Hospital.

The memory of Dr. W. W. Duke was honored by Dr. Dan H. Campbell, Associate Professor of Immunochemistry, California Institute of Technology, who discussed "The Immunological Aspects of Allergy."

The big social event, the cocktail party followed by a dinner dance and floor show, probably will not be surpassed until we meet again in St. Louis.

Even more enlightening, and interesting to many, were the extrasession informal groups gathering by accident or by design in private homes, rooms, or hotel lobby. These had an enthusiasm and good fellowship reminiscent of prewar spirit. Some familiar

faces were missed with sadness. Newer ones, however, attest to a continuation of delightful sessions at future meetings.

The next meeting will be held in Atlantic City, December, 1948.

ALAN CAZORT.

HONORARY FELLOWSHIP

Gilman, Alfred, Professor of Pharmacology, Columbia University, New York, N. Y.

DIRECT FELLOWSHIP

Kremer, Willem, Emmastrart 26-28, Amsterdam, Holland.

ADVANCED TO FELLOWSHIP

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Lieder, Louis E., 327 Osborn Bldg., Cleveland 15, Ohio.
Lowance, Mason I., 478 Peachtree St., Atlanta, Ga.
Mauser, Carl L., 400—29th St., Oakland, Calif.
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ANNOUNCEMENT

The Milwaukee County Medical Society is now Executive headquarters for the Academy of Allergy. The address is 208 East Wisconsin Ave., Milwaukee 2, Wis. Mr. J. O. Kelley is Executive Secretary of the Milwaukee County Medical Society. Academy affairs will be taken care of by Mr. A. H. Luthmers, who is assistant to Mr. Kelley. Executive headquarters will take care of the clerical work heretofore done by the Academy's secretary and the Academy's treasurer.